A STUDY ON ROTAVIRUS GASTROENTERITIS IN CHILDREN UNDER FIVE YEARS IN COIMBATORE.

Dissertation submitted in

Partial fulfillment of the Regulations required for the award of

M.D. DEGREE

In

MICROBIOLOGY– BRANCH IV

The Tamil Nadu

DR. M.G.R. MEDICAL UNIVERSITY

Chennai

APRIL 2017.

CERTIFICATE

This is to certify that the enclosed work "**A STUDY ON ROTAVIRUS GASTROENTERITIS IN CHILDREN UNDER FIVE YEARS IN COIMBATORE"** submitted by **Dr.R. Senthilkumar** to The Tamilnadu Dr.M.G.R. Medical University is based on bonafide cases studied and analyzed by the candidate in the Department of Microbiology, Coimbatore Medical College Hospital, during the period from July 2015 to June 2016 under the guidance and supervision **Dr.N. Mythily M.D.**, Professor**,** Department of Microbiology.

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I, Dr R. SENTHILKUMAR solemnly declare that the dissertation entitled **"A STUDY ON ROTAVIRUS GASTROENTERITIS IN CHILDREN UNDER FIVE YEARS IN COIMBATORE**" was done by me at Coimbatore Medical College Hospital, during the period from July 2015 to June 2016 under the guidance and supervision of **Dr.N.MYTHILY, M.D.,** Professor, Department of Microbiology, Coimbatore Medical College, Coimbatore.

This dissertation is submitted to The Tamilnadu Dr.M.G.R. Medical University towards the partial fulfillment of the requirement for the award of M.D Degree (Branch - IV) in Microbiology.

I have not submitted this dissertation on any occasion to any University for the award of any degree.

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ACKNOWLEDGEMENT

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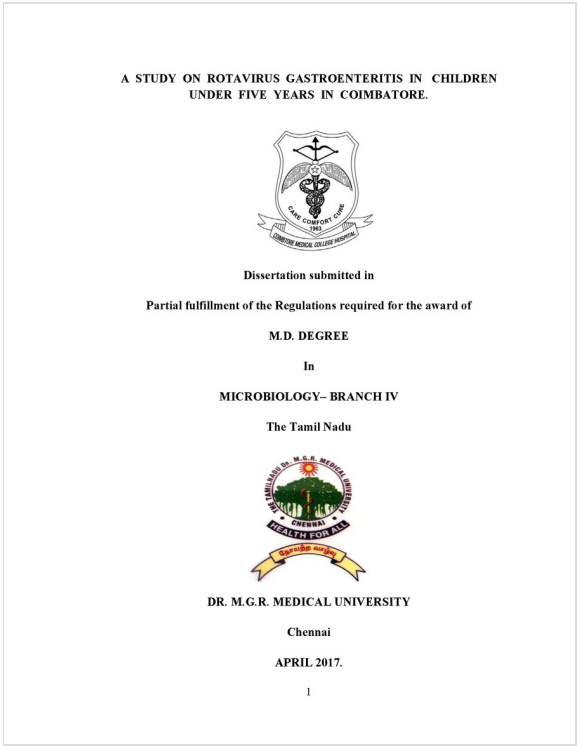

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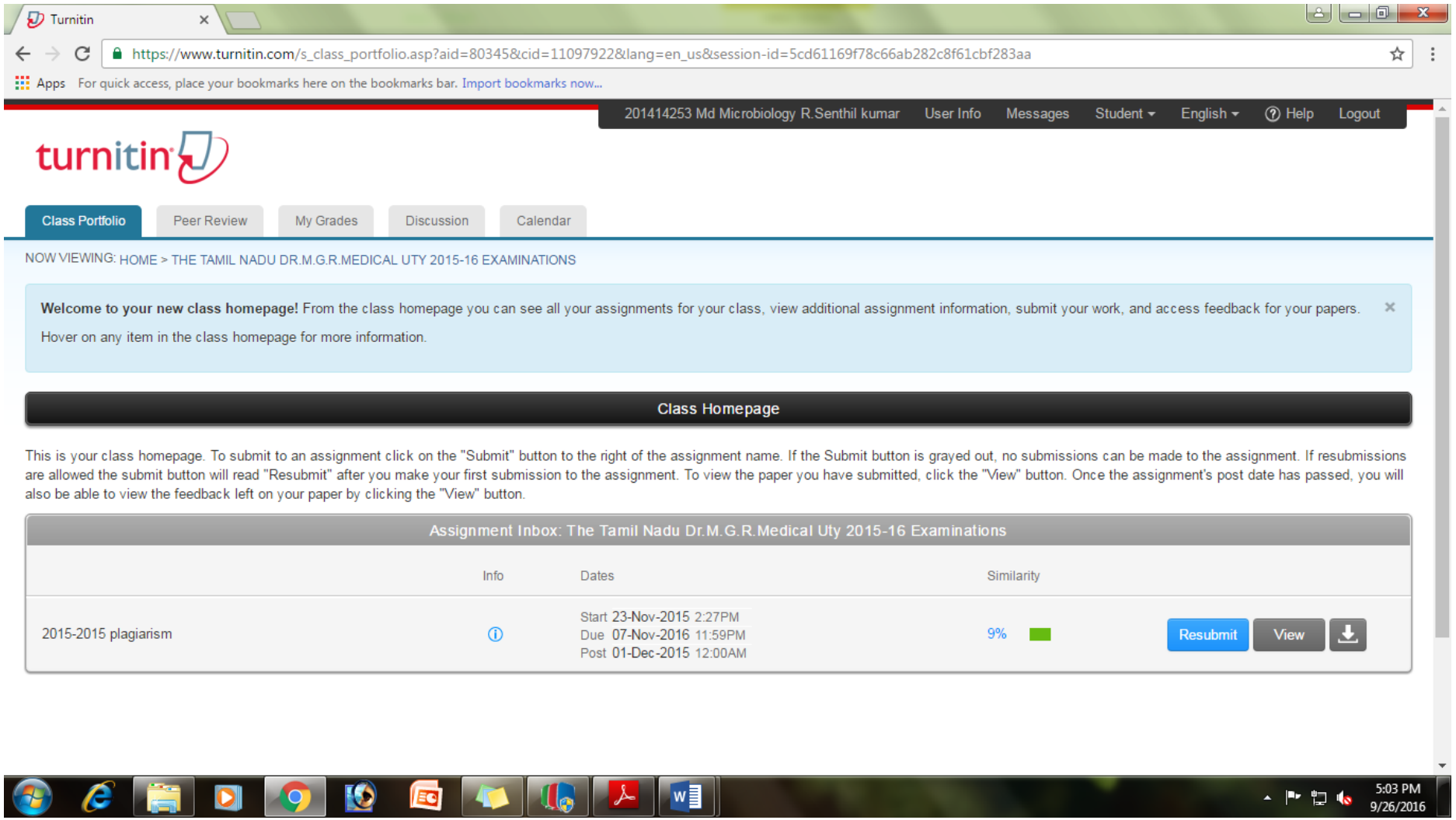
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LIST OF ABBREVIATIONS

AGE	Acute gastroenteritis
DLP	Double Layered Particles
EIA	Enzyme Immunoassay
ELISA	Enzyme Linked Immunosorbent Assay
EM	Electron Microscope
E-type	Electropherotype
IAP	Indian Academy of Paediatrics
ICG	Immunochromatography
LA	Latex Agglutination
NIH	National Institute of Health
NSP	Non Structural Proteins
ORT	Oral Rehydration Therapy
RVGE	Rotavirus Gastroenteritis
RT-PCR	Reverse Transcryptase Polymerase Chain Reaction
SG	Sub Group
SLP	Single Layered Particles
ssRNA	Single Stranded RNA
TLP	Triple Layered Particulars
VP	Viral Proteins
WHO	World Health Organisation

INTRODUCTION

INTRODUCTION

Rotavirus remains the world's **commonest cause of gastroenteritis** among children predominantly in **developing countries**. Globally rotavirus alone is responsible for around 139 million cases or almost 40% of gastroenteritis related hospitalization, in the paediatric age group every year. The impact of Rotavirus infection in children is far greater in **India** accounting for about 26 % of all gastroenteritis related hospitalization among children.

In addition, diarrhoea plays a major role in **malnutrition** among children which predisposes them to other infectious diseases and increases childhood morbidity and mortality. Rotavirus infects almost all children at least once by the age of five years. It is prevalent in all geographical regions of the world and almost all socioeconomic groups.

Children attending daycare are at a higher risk of hospitalization due to Rotavirus infection. Asymptomatic infection in newborn nurseries occurs round the year and infection can spread to families causing gastroenteritis in adult caregivers of children which might be the reason for endemic cases.

Though the Rotavirus infection is self-limited, it can cause **severe dehydration** and **electrolyte imbalance** leading to complications such as shock, cardiac failure, seizures and aspiration of vomitus resulting in aspiration pneumonitis. Serologic evidences indicate that frequent rotavirus infection leads to

development of coeliac disease in genetically predisposed children. It can also cause chronic diarrhoea in immuno compromised children and relatively severe gastroenteritis in adults with immuno suppression. Studies indicate that Rotavirus accounts for 4-7% of **traveler's diarrhoea.**

However, the significance of the rotavirus gastroenteritis is said to be under estimated in our country since most of the children with symptoms of gastroenteritis are not brought to the health care set-up for treatment. Routine screening for Rotavirus infection is also not being done in symptomatic children.

The word '**Rota' means wheel** due to its distinct wheel like shape. It was discovered in Nebraska in United States among '*Calves*' in 1969 and first human cases were described in 1973 in children with acute gastroenteritis.

Rotavirus belongs to the **family Reoviridae** and the virion is a non-enveloped icosahedral particle. It consists of three concentric protein layers, which encloses 11 segments of ds-RNA together with polymerase [VP-1] and capping enzyme [VP-3] complexes. These segments encode six structural proteins VP-1 to VP-4, VP-6 and VP-7 and six non-structural proteins NSP-1 to NSP-6. The innermost layer is VP2 which encloses two proteins VP-1 and VP-3. The middle layer is VP-6 and though not exposed on the viral surface, VP-6 is the target of the most abundant antibodies produced by Rotavirus infection. The outermost layer [glycoprotein] is made up of VP-7 and VP-4 spikes which protrude from the virion. VP-4 is the major cell attachment protein and virulence determinant.

Based on the VP-6 capsid gene, the virus has been classified into seven major **genogroups A to G**; among them Group A, B and C infect human beings. Group A is most commonly detected among children with endemic gastroenteritis. Group B and C are associated with epidemics of gastroenteritis affecting all age groups. The strain ADRV - **Adult Diarrhoea Rota Virus of Group B** has been linked to large **outbreaks** of severe diarrhoea in China as well as smaller outbreaks and endemic disease in Bangladesh and India. Group C causes less severe gastroenteritis in both adults and children. Serotype-G2 predominates in outbreaks of Group A rotavirus gastroenteritis among adults and is found to be particularly virulent.

On the basis of outer shell proteins VP-7 and VP-4, the Group A viruses are further classified into 27 **G and 35 P genotypes** respectively. Worldwide the most prevalent genotypes causing majority of infections are **G1P[8], G2P[4], G3P[8], G4P[8], and G9P[8].**

For efficient rotavirus infection, priming of the virus by intestinal trypsin is necessary. It cleaves the spike protein,VP-4 into an aminoterminal fragment VP-8 and carboxy terminal fragment vp-5 .The VP-5 fragment involves in the binding of virus with the intestinal epithelial receptor possibly an '***intergrin***'. The pathogenesis of rotavirus is complex and multifactorial. The possible potential role of the virus being enterotoxin, malabsorption [sodium and glucose] related to mucosal damage of intestinal epithelial cells, depletion of disaccharidases ,etc.

Immunity in Rotavirus infection is complex; Innate, cellular and humoral mechanisms participate in elimination of infection. The humoral immunity plays a major role in protection from severe disease. Protection of Rotavirus infection after natural infection or immunization is more reliable against viruses of the same serotype as the immunizing strain. The duration of immunity to Rotavirus is limited; repeated symptomatic infections commonly occur in both children and adults, but generally with less severity. On the other hand, cross protection between serotypes occurs after multiple infections.

In Rotavirus reinfection is common. Asymptomatic infections are common in infants below 6 months of age; the time during which the maternal antibody confers protection. Such infections protect against severe infection but do not offer protection against reinfection. Young children can suffer up to five re-infections within 2 years of age and 90% of children by the age of three years show antibodies to rotavirus. Local immune factors like **secretory IgA** and **interferon** also play an important role in protection against rotavirus infection.

From some clinical studies of Rotavirus infection, breast feeding was found to be associated with lesser frequency of vomiting and less severe dehydration with a lower risk of hospitalization.

Rotavirus shows an **unusual genomic diversity** worldwide. During the Rotavirus outbreaks, multiple strains which show combination of several G and P types are seen.**G1,G2,G3 and G4 strains account for 97.5**% of all rotavirus

infections in Asia, North America, and Europe.G5,G8,G9 and G12 strains are prevalent in several areas and G9 strains occasionally show predominance. Epidemiologically G1 strain is most commonly prevalent worldwide followed by G9 and G2. Children infected with G1 strains have a greater risk of developing gastroenteritis with severe dehydration than those infected with other strains of Rotavirus.

Rotavirus gastroenteritis shows **marked seasonal variation**. In temperate countries epidemic peak occurs only in winter whereas in India it occurs throughout the year but more during November to February.

Rotaviruses are **highly infectious** (even <100 viruses can cause infection); the best documented mode of viral transmission is **faeco-oral-route**. Rotaviruses are stable in environment and they can survive on toys, books, clothes and surfaces for a longer period. Perhaps they are relatively resistant to most soaps and disinfectants; so there is a possible spread of infection through Health care staff and caregivers of the children especially when proper hand hygiene is not being followed.

Rotavirus shows variation in prevalent strains from time to time and region to region. Genetic re-assortment is more common due to antigenic drift; occasionally **genogroup re-assortment** between human and animal strains has been observed. This contributes to a continuous introduction of genetic diversity

which necessitates the ongoing surveillance to assess the efficacy of rotavirus vaccine

Rotavirus gastroenteritis cannot be eliminated through improvement in sanitation alone since there is a possible spread of virus through, aerosols, vomitus and contact with fomites and environmental contamination. Moreover there is **no specific treatment** for Rotavirus infection except supportive measures such as rehydration with ORS/IVF; so it is necessary to develop a safe and effective vaccine to decrease the occurrence and severity of rotavirus gastroenteritis in the paediatric population.

The **Prophylactic Rotavirus vaccination** within the perspective of National Immunization Program in developed countries has been associated with significant reduction in mortality and morbidity as well as hospitalization among children under five years.

Considering the severity of Rotavirus infection and its associated mortality and morbidity in children under five years, as well as the importance of genetic re-assortment, this study is undertaken to assess the prevalent genotypes of Rotavirus responsible for acute gastroenteritis within the time period in Coimbatore.

AIM & OBJECTIVES

AIM AND OBJECTIVES

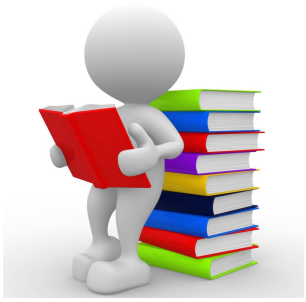
AIM

To analyse in detail about the prevalence, demographic profile, seasonal trends and detection methods of Rotavirus Gastroenteritis in children under five years in Coimbatore.

OBJECTIVES

1. To detect the Rotaviral antigen from stool specimen in children under 5 years with acute gastroenteritis by ENZYME IMMUNO ASSAY.
2. To compare Enzyme Linked Immunosorbent Assay (ELISA) and Immunochromotography (ICG) in detection of rotaviral antigen from stool specimen.
3. To analyse in detail about the demographic profile and seasonal trends of Rotavirus diarrhea in our place.
4. To study the proportion of G/P types among positive cases and their association with the clinical severity of Rotavirus diarrhea.

REVIEW OF LITERATURE



REVIEW OF LITERATURE

HISTORY

In 1963, the virus with distinct morphologic features was first observed by **Adam and Kraft** by electron microscopy in small intestine and rectal swab specimens from mice and monkeys. These agents were called as **infant mice virus and simian agent 11** accordingly which were later identified as Rotaviruses. They were described as 70nm particles with a unique morphology (Latin word-Rota meaning wheel) - "***wheel like appearance"*****[1].**

In 1969, these virus particles were demonstrated in the stool sample of calves' with diarrhea, thus relating these virus particles with diarrhoeal disease in cattle [2].

In 1973, the association between these calf viruses and human diarrhoeal disease were documented (Bishop et al., 1973). They demonstrated the Rotavirus particles from duodenal mucosa biopsy specimens and later in stool specimens of children with acute gastroenteritis in Melbourne, Australia (Flewett et al, bishop et al).

Later it was found that the human Rotaviruses and the animal Rotaviruses shared a group antigen and they were classified as members of Rotavirus genus within the family Reoviridae [1].

In 1980, the virus particles were morphologically indistinguishable from the established Rotavirus strains except lacking the common group antigen which was discovered in pigs. This led to the identification of six additional Rotavirus groups (B to G) based on a common group antigen, with previous Rotavirus strains classified as group A[2]. Group A, B, and C have been associated with human diseases while other groups have been linked with diseases of animals [3].

EPIDEMIOLOGY

Acute gastroenteritis is one of the most common illnesses of humans and its morbidity and mortality are higher among children and the elderly [Wilhelmi et al 2003]. Rotavirus is the important causative agent of diarrhoea with severe dehydration in children in developed as well as developing world [Alrifai et al 2009]. About four billion episodes of diarrhoea occur every year worldwide, of which more than 90% are from developing countries [7]. According to the Global Burden of Disease Study 2010, Diarrhoea is in the fourth common place in terms of maximum Disability Life Years (DALY) lost.

Rotavirus is the commonest etiological agent of severe diarrhoea in infants and young children worldwide [4] and accounts for 40% of gastroenteritis related hospitalization [19]. In India, 30% of hospitalized diarrhoeal cases are attributable to Rotaviruses [5].

In developing countries, Rotavirus alone is responsible for around half a million deaths annually. In India, among more than 2.3 million annual deaths in children [4], diarrhoea is the third leading cause of childhood mortality and accounts for about 3,34,000 deaths[5].

In addition, diarrhoea plays a major role in malnutrition among children and predisposes them to other infectious diseases which leads to increase in childhood morbidity and mortality [7].

ETIOLOGY

Diarrhoea may be caused by Bacteria, Viruses and Parasites and rarely Fungi [8]. Among them viruses are the major etiological agents contributing to 70% of all diarrhoeal cases worldwide (Webb and Star, 2005).

Viruses causing diarrhoea in children :

Rotavirus, Adenovirus, Astrovirus , Calcivirus (Norovirus & Sapovirus), Coronavirus and Torovirus[8]

ADENOVIRUS

- They are non-enveloped ds-DNA viruses approximately 70-90 nm in diameter; spherical in the form of icosahedran
- Adenovirus can cause **epidemic** and **sporadic** diarrhoea in infants and outbreaks are associated with **type 3&7** infections

- **Enteric adenovirus** types **40&41** account for about **5-15%** of all diarrhoeal cases in children[9&10]

ASTROVIRUS

- They belong to the family ***Astroviridae*** and the genome is positive sense ss-RNA. Under electron microscope, the virus has a ***star like*** morphology.
- Human viruses are classified in the ***Mamastrivirus*** genus of family Astroviridae; eight serotypes have been identified so far; among which, **serotype-1** is the most common.
- Astroviruses are linked with epidemics in paediatric wards, day care centers and nursing homes and contributes **7-15%** of diarrhoeal cases especially in children less than 1 year[10].

CALCIVIRUS

- The virion particles are small (27-40nm in diameter), non-enveloped of icosahedral symmetry with 32 cup shaped depressions on the surface
- In human infection 2 genera of calcivirus have been linked:
  1. Norovirus
  2. Sapovirus

NOROVIRUS

- They are small *round structured* virus which are the major cause of **non-bacterial epidemic gastroenteritis**[10]
- It includes Norwalk and Snow mountain viruses

NORWALK VIRUS

- They are highly infectious and commonly associated with **nosocomial infections**[11]
- It affects older children and adults

SNOW MOUNTAIN VIRUS

- Epidemic gastroenteritis in children and adults were recorded and shown to be due to snow mountain virus[12]

SAPPOVIRUS

- It was first identified in *Sapporo, Japan* and hence the name (27-40 nm in diameter)[13]
- Causes sporadic cases and occasional outbreaks of diarrhoea predominantly among *closed population* in infants, young children and elderly [13].

CORONAVIRUS

- The name corona is used because of *crown* like projections on the surface under electron microscope with helical nucleocapsid of 120-160 nm in diameter[14]
- They are linked with diarrhoeal cases in paediatric age group[29]

TOROVIRUS

- They are enveloped positive strand RNA viruses, approximately 100-140nm and associated with ***hospital acquired gastroenteritis*** in paediatric patients[15]

ROTAVIRUS

It is well documented that Rotaviruses are the major cause of diarrhea in human infants, young calves and piglets [16]. Rotaviruse is the agent of infantile diarrhoea of human, epizootic diarrhoea of infant mice, Nebraska calf diarrhoea and SA 11 virus of monkeys [17].

Despite gains in controlling diarrhoeal deaths, the disease burden still remains high. Studies indicate there is an approximately 139 million cases of gastroenteritis in children worldwide (Institute of Medicine, 1986). Although the prevalence of Rotavirus diarrhea is similar in the developing as well as developed countries (Alrifai et al.,), mortality is comparatively

higher in developing countries which accounts for around 6,11,000 deaths in children less than five years[17].

There is widespread distribution of Rotavirus in the community. Almost all children have at least one episode of Rotavirus infection within 5 years of age [16]. The high prevalence of Rotavirus is due to persistence of subclinical infection throughout the lifetime.

- A study done in Washington over a period of 8 years shows 34.5% of 1537 children found to be Rotavirus positive in their stool specimens.
- A study done in Japan over a period of six years showed 45% of 1910 children to be positive for Rotavirus from stool specimens.
- An Australian study showed 39.6% of positivity for Rotavirus in stool samples, among 3785 children with acute diarrhoea.
- Hospital based studies in Africa, Asia and Latin America report 25%-55% of hospitalization in children due to Rotavirus infection.
- In countries of the eastern Mediterranean region, studies conducted among children with acute diarrhoea shows Rotavirus in 40% of inpatients and 23% of outpatients by the age of 3 years.
- In a population based study among infants and young children in Saudi Arabia, Rotavirus was detected in 95% of stool specimens. In

Jordan, in hospitalized children below five years, Rotavirus was detected in 39.8% of stool specimens.

- In India, a health care facility-based study of children has exhibited Rotavirus infection to account for around 26% of all diarrhoea-related hospitalization [18].

Primarily Rotavirus infection in younger children between 6 months to 2 years of age is about 55% whereas it is 18.7% in children less than 6 months and 16% in the age group of 5-13 years. During the first five years of life, almost every child acquires rotavirus infection.

The prevalence of Rotavirus infection is similar both in developing as well as developed countries before vaccination [15]. Although the rotavirus vaccines have been implemented in many middle income countries in Central and South America, vaccines are not yet available in countries of Asia and Africa. Widespread vaccination would markedly lower the global mortality and financial burden due to Rotavirus infection.

Estimates indicate, there is an approximately 1,22,000, 27,000 and 2,000 deaths related to Rotavirus infection in India, China and Pakistan respectively.

Adult contacts may be infected, as evidenced by seroconversion [28], but they rarely exhibit symptoms, and virus is infrequently detected in their

stools. However epidemics of severe disease have been recorded in adults, particularly in closed populations, such as in geriatric ward, healthcare workers, travelers, military people and parents of infected children [1].

SEROTYPES OF ROTAVIRUS

The serotype classification is based on the surface protein vp7 (G-type) and VP4 (P-type) which are the target of neutralizing antibodies [20]. There are 25 different G- serotypes and 35 P serotypes found at present among group A Rotavirus of humans and animals. The prevalent G and P type combination in population is somewhat lower than the expected number of G and P type combination. Approximately, 12 G type (1 to 6, 8 to 12 and 20) and 15 P-types (1 to 6, 8 to 11,13,14,19,25 and 28) have been linked with human infection[10].

The global distribution of human Rotavirus serotype appears to be consistent. Since, 1989 till now, serotypes G1,G2,G3 and G4 constantly make up the predominant strains detected especially in developed countries.

In 124 studies [1989-2004] on the global Rotavirus Serotypes and Genotypes distribution, G1 strains were the most commonly detected among 45,571 typed strains [30]. Despite this, in the last decade G9 strains have emerged as a predominant serotype in some countries such as Australia,

United States, Ghana, India and Brazil, in some parts of Africa, G8 strains have emerged as predominant strains.

Initially, serotype analyses of clinical isolates for their P types (VP4) were not widely performed because of the lack of appropriate mono specific serologic reagents. However, RT PCR for P (VP4) genotype had made it possible. Based upon the segment analysis of VP4, P1A[8] and P1B[4]strains are the most frequently detected strains in humans. The P1A[8] genotype is almost always found in association with G1,G2,G3,G4 and G9 strains whereas P1B[4] genotype characteristically has specificity with serotype G2.

In the review of 16,474 strains evaluated so far, both VP4 and VP7 specificities indicate that the predominant P-G combinations, G1P[8], G2P[4], G3P[8] and G4P[8] contribute 88.5% of all the serotypes and recent evidence suggests that G9 P[8] strains are also detected in humans frequently[20].

It is assumed that the studies on serotypic classification gained much attention because such classification will be relevant and correlate well in relation to protective immunity in Rotavirus infection. The recent documentation provides that G and P monotype vaccine is associated with efficient protection against all Rotavirus infections [5].

In humans, the heterotypic immunity plays a major role in the epidemiology of Rotavirus infection [5]. In spite of the potential serotypic

diversity of Rotavirus, immunity appears rapidly after limited exposure only to a restricted number of serotypes.

SUB GROUPS

Group A has 4 sub-groups based on the vp5 diversity.

SG1, SG2, SG1 &2 and NON1 & NON2

SG1, SG2 possibly infect humans [21].

GENOGROUPS

Three genogroups (two major & one minor) have been established in human strains by the RNA-RNA hybridization technique using the reference strain WA DS-1 and AV-1. Human Rotavirus belongs to the WA genogroup, have origin with porcine Rotavirus and those belonging to DS1 genogroup, have origin with bovine Rotavirus [22].

ELECTROPHEROTYPE (E-TYPE)

Two major electropherotypes, '*long' and 'short'* have been demonstrated by separation of Rotavirus genome using polyacrylamide gel electrophoresis [23]. The '*long'* pattern corresponding to the sub group I and the '*short'* pattern corresponding to the sub group II of the Group A human Rotavirus [24].

ROTAVIRUS INFECTION IN ADULT

Adults get frequent reinfection with Rotavirus but are asymptomatic or affected less severely; subclinical infections are most common among them. Outbreaks in adults have also been described; associated with 4-7% of traveler's diarrhoea [25].

Symptomatic Rotavirus gastroenteritis was found in some adults undergoing bone marrow transplantation and patients with malignant disease undergoing chemotherapy due to immunosuppression.

Group B-Rotavirus (Adult Diarrhoea Rotavirus-ADRV) have been implicated in several large outbreaks involving up to 20,000 individuals of severe gastroenteritis in adults in China[29]; also in smaller outbreaks and endemic cases in Bangladesh and India[25]. Group B- Rotavirus (Group B-RV) infection occurs primarily as epidemics or sporadic cases, possibly due to contamination of water sources.

NOSOCOMIAL INFECTION

Rotavirus often causes Hospital acquired infection. Nosocomial outbreaks have been recorded in newborn nurseries in Italy and neonatal ICUs in United States. Such infections mainly involve young infants (0-5 months of age) [25].

TRANSMISSION OF ROTAVIRUS

The best documented mode of Rotavirus transmission is faeco-oral route [26]. Rotaviruses are highly contagious; stable in ambient temperature and needs low dose for human infection (<100 virus particles is enough to cause infection) [27]. The source of infection for the young infant is either from older sibling or patient with subclinical infection. There has been speculation about Rotavirus transmission by respiratory route but it is not the usual mode of transmission [25].

Community wide water borne epidemics of group A Rotavirus (G-A RV) have been documented in Turkey in 2011. Effective disinfection of contaminated material and proper hand washing are the most important measures to control Rotavirus infection especially in hospitals and institutions [1].

VIRAL SHEDDING

Virus is shed in very large amount in faeces (10^{12} particles per gram faeces)[29]. The duration of viral shedding may persist upto days and is prolonged in immuno-compromised children [29].

RESISTANCE OF ROTAVIRUS

Rotaviruses are stable at 50 deg C, pH range 3-9 and to some lipid solvents like ether, chloroform but inactivated by 95% ethanol, phenol and chlorine [29].

INCUBATION PERIOD

The average incubation period is 1-3 days [29].

DISTRIBUTION

Rotavirus is distributed throughout the world and the major causative agent of severe diarrhoea with dehydration in infants and young children. According to WHO reports, there are 2 billion cases of diarrhoeal disease every year globally in children less than five years [31]; it also estimated that 85% of global Rotaviral deaths occur in Africa, Asia and Latin America [32].

SEASONAL VARIATION

Before the introduction of vaccination, Rotavirus showed marked seasonal variation in developed countries with the epidemic peaks occur in the cooler month of the year [29]. The cause for this seasonal pattern is not known. Following the introduction of Rotavirus vaccination the seasonal pattern has decreased markedly.

On the other hand in the tropics, no or minimal seasonal variations has been noted [33-35]. This may be due to high birth rates as well as high transmission rates found in developing countries.

AGE, SEX AND SOCIOECONOMIC STATUS

In developed countries, Rotavirus gastroenteritis most frequently affects infants and young children between 6 months to 2 years of age[29] followed by the infants younger than 6 month of age. Male children were found to be twice as susceptible and more likely to be hospitalized than female children [1]. Living in crowded condition may predispose them to earlier Rotavirus infection.

ANIMAL SUCEPTIBILITY

- Rotaviruses have a wide range of hosts such as mice, calves, swine and piglets
- Most isolates have been recovered from newborn animals with diarrhoea
- Cross-species infections can occur in experimental inoculations [19]
- Newborn animals often exhibit subclinical infection, reflecting the presence of maternal antibody; overt disease is more common during the weaning period [19]

PROPAGATION IN CELL CULTURE

- Rotaviruses are fastidious in nature and difficult to cultivate
- Most of the group A human Rotaviruses can be cultivated if it is pretreated with a proteolytic enzyme like trypsin[19]

MALNUTRITION

Malnutrition plays an important role by increasing the severity of Rotavirus gastroenteritis [36]. Repeated diarrhoeal infection may be a precipitating factor leading to the development of malnutrition as the infection damages the intestinal mucosa and absorptive cells are being lost over a period.

MOLECULAR EPIDEMIOLOGIC STUDIES

The gel electrophoresis is the method followed very earlier to study the molecular epidemiology of human Rotavirus [29]. Molecular epidemiological studies have analyzed isolates, based on differences in the migration of the 11 segments of ds-RNA genome in polyacrylamide gel electrophoresis. The differences in electropherotypes can be used to differentiate group A viruses from other groups [3]. But they cannot be used to predict serotypes. Later, RNA/RNA hybridization techniques were developed which employs labeled ss-RNA viral transcripts as probe for genetic ds-RNA. These studies identified two major genogroups (family) of

human Rotavirus and showed that human strains lack homology with animal strains [3]. Recent advances replaced the electrophoretic and RNA/RNA hybridization techniques.

MORPHOLOGY OF ROTAVIRUS

The Rotavirus has a distinct morphologic feature and three types of particles can be observed under electron microscopy.

- The complete particle appears like a '*wheel with spokes'* and a well-defined smooth outer rim [1].
- The complete virion is also called as triple layered particle (**TLP**)
- The virion lacking the outer shell are called as double layered particle (**DLP**); they are also called as 'rough particles' because their peripheral trimeric subunits of the inner capsid project outside
- Single layered particles (**SLP/core**) are seen less often. They lack genomic RNA and are aggregated [25].
- The rotavirus virion is a non-enveloped icasohedral particle, size approximately 770 Å in diameter excluding the vp4 spikes belong to Reoviridae family [21]. It consists of 3 concentric protein layers which envelop segmented ds-RNA together with polymerase and capping enzyme complex .This segments encode six structural proteins (VP-1 to VP-4, VP-6 and VP-7) and 6 non-structural proteins (NSP1 to NSP6) [22].

- The innermost layer consists of 120 copies of VP-2 arranged in one icasohedral lattice. The VP-1 polymerase and VP3 capping enzyme are anchored inside VP-2 shell adjacent to the pores at the icasohedral vertices [37].
- The middle layer consists of 780 copies of VP-6 which forms the thick trimeric pillars in the icasohedral lattice [3].
- The VP-6 although not exposed on the viral surface, is the target of the most abundant antibodies produced by Rotavirus infection [3].
- The genome VP-1 and VP-3 together with inner protein layer make up the transcriptionally active double layered particle (**DLP**).
- The outermost layer is thin, and it consists of 780 copies of a coat glycoprotein VP-7, and sixty vp4 spikes which protrude from virion [21].
- The VP-4 and VP7 translocate the DLP across a host cell membrane and into the cytoplasm [21].
- VP4 is the major cell attachment protein and virulence determinant and growth restriction factor in cell culture [17].
- During the entry into a host intestinal cell, the viral proteins vp4 and vp7 are shed from the virion which in turn leads to activation of the viral polymerase [21].

ROTAVIRUS PROTEINS AND FUNCTIONS [2, 38]

- VP 1 is a core protein, which act as RNA dependent RNA polymerase; helps in SS-RNA binding and couples with VP 3
- VP 2 is present in core, with the major function includes RNA binding and required for replicase activity of VP 1
- VP 3 is located in core, activates Guanyl transferase and methyl transferase
- VP 4 is situated in the outer capsid. It helps in host cell attachment; It causes Haemagglutination and produce neutralizing antibodies
- VP 6 is present in inner capsid, required for transcription
- VP 7 located in the outer capsid, produces neutralizing antibodies
- NSP 1 (NS 53) is a Non-structural protein, it promotes RNA binding
- NSP 3 (NS 34) ,a Non-structural protein, helps in RNA binding; stimulates NTPase and Helicase activity
- NSP 4 is a Non-structural protein, involved in accumulation of intracellular Calcium, RNA replication, and enterotoxin production.
- NSP 5 - Non-structural protein, helps in RNA binding; activation of protein kinase and interacts with VP 2 and NSP 6
- NSP 6 - Non-structural protein, interacts with NSP 5

PATHOGENESIS

The pathogenesis of Rotavirus is complex and incompletely understood. The virion infects villous enterocytes and causes cell destruction. The release of progeny takes place by cell lysis [29].

For efficient Rotavirus infection to occur, priming of virus by intestinal trypsin is necessary which cleaves the spike protein VP-4 into two fragments namely VP-8-aminoterminal and vp5-carboxyterminal [38]. The vp8 contains haem-agglutination domain which forms the spike heads and vp5 contains a membrane interaction domain which forms the spike body. Cleavage and rearrangement of vp4 increases the rigidity of VP4 spikes [29].

VIRAL ENTRY AND REPLICATION

The cell entry pathway of Rotavirus involves virus interaction with host cell surface. Rotavirus strains related with non-human animals, attaches to host cell through vp8 fragment which bind neuraminidase-sensitive sialic acids on cell membrane glycoprotein or glycolipids [38].

Rotavirus strains linked with human infection, bind an alternative receptor possibly an Integrin using the VP5 fragment. During cell entry the VP5 undergoes a significant fold back re-arrangement, translocating its hydrophobic apex from one end to the other end of the molecule [39].

After attachment and penetration, un-coating of virus particles takes place in lysosomes of the cell cytoplasm. The outer cell of virus is removed and core associated RNA transcriptase is activated. The transcriptase transcribes mRNA molecules from negative strand of ds-RNA segment [29].

The core contains all necessary enzymes for transcribing, capping and extruding the mRNA from the core and leaving the ds-RNA genome segments inside [29]. After being extruded from the core, the mRNA is translated into primary gene products. A viral replicase is responsible for synthesizing negative sense strands to form the double strand genome segments and this process take place in partially completed core structures [29].

The mechanism of assembly of the correct compliment of genome segments into a developing new viral core is probably through the viral polypeptides which may self - assemble to form the inner and outer capsid shells [29].

Rotavirus produces inclusion bodies in the cytoplasm. Rotavirus morphogenesis involves budding of single shelled particles into the rough endoplasmic reticulum. The pseudo envelopes are then removed and outer capsids are added. This distinct pathway is used because the major outer capsid proteins of Rotavirus are glycosylated [29].

The possible role of Rotaviruse in pathogenesis of gastroenteritis:

1. Viral enterotoxin [41]

2. Malabsorption due to intestinal mucosal damage [40]

3. Depletion of the enzyme 'disaccharridases' [38]

In Rotavirus infected pigs, study of duodenal biopsy specimen by light microscopy revealed, shortened and blunted villi with cuboid epithelium, **crypt hypertrophy** and mononuclear cell infiltration in lamina propria [38].

NSP4 – A '**viroporin**' is a novel protein which mediates cell death by increasing intracellular calcium and affecting plasma membrane permeability as well as tight junctions of villous cells. It is a viral enterotoxin which increases the enteric secretion of fluids through a 'signal transduction pathway [29].

Rotavirus reduces the host protein synthesis through NSP3 which is shown by marked decrease in the level of host cell protein and increase in the level of viral protein[38]. Rotavirus NSP1 interacts with IRF3 (**Interferon Regulatory Factor**) and leads to degradation of IRF3 which interferes with the innate host immune mechanisms [3].

Rotavirus infection causes alterations in the tight junctions of the small intestinal epithelial cells. The intestinal epithelial cells are sloughed off due

to loss of tight junctions during the period of viral replication which leads to decreased glucose and sodium absorption [38}.

The effect of Rotavirus infection in human intestinal epithelial cell lines shows that the following factors are involved: [38]

1.Interleukin-8

2.Growth related peptide-2

3.Osteopontin

4.Chemokine

Of which, the chemokine secreted by infected enterocytes plays an active role in pathogenesis of Rotavirus gastroenteritis

DIVERSITY OF ROTAVIRUS [41]

The Mechanism Involved In Diversity Of Rotavirus Includes The Following: [20]

1. *Point mutation accumulation* leads to formation of new genetic lineages and emergence of mutants which helps virus to escape from humoral antibody response
2. *Genetic re-assortment* between two different human Rotavirus strains infection at a time
3. Possible genetic re-assortment of human Rotavirus strains among animal strains contributing to diversity.

IMMUNITY

The immune response to Rotavirus infection is complex and multi factorial: Innate, cellular and humoral immunity contributes to eliminate the infection. Humoral immunity has a dominant role among them to prevent severe disease on successive infection or on primary infection after immunization [29].

Cross protection between different serotypes occurs after successive infection. The duration of immunity against Rotavirus infection is limited; reinfection occurs both in children and adults but generally with less severity [38].

Generally reinfections are common in Rotavirus disease. Asymptomatic infections are frequently found in infants below the age of six months, the time during which the maternal antibody confers protection. Such Neonatal infection does not protect against reinfection but protects against severe disease [29].

Young children can suffer up to five re-infections by the age of two years and 90% of the children show antibodies against Rotavirus by the age of three years [29]. Local immune factors such as secretory IgA or Interferon plays an important role in protection against Rotavirus infection [29].

The role of breast feeding in protection of humans against Rotavirus gastroenteritis is obscure. A hospital based study done in Bangladesh among

children admitted with Rotavirus gastroenteritis suggests that breast feeding prevents Rotavirus less effectively than gastroenteritis caused by other agents [42]. However, it is noted that, in some clinical studies of Rotavirus infection, breast feeding is linked with less frequency of vomiting and less severe dehydration as well as lower risk of hospitalization [28,4].

Neutralizing monoclonal antibodies that recognize the vp8 fragment of vp4, blocks the attachment of sialic acid dependent Rotavirus to host cells and antibodies that recognize VP5 fragment of VP4 blocks post binding entry events. Antibodies against the VP7 protein blocks virion un-coating. **IgA monoclonal antibodies** that recognize the **middle layer of protein VP6** does not neutralize the virions but blocks transcription by DLPs and protect the mice from Rotavirus infection as found in vitro studies [42].

CLINICAL FINDINGS

Rotavirus is responsible for the major cause of diarrhoeal diseases in children below five years. The classical clinical features include watery diarrhoea, fever, vomiting and abdominal pain. Infected children may also have cough or running nose [27,33,41]

Rotavirus gastroenteritis is also called "**Winter vomiting disease"** since it is associated with combination of vomiting and seasonal trends in winter months [44].

Diarrhoea along with vomiting rapidly results in severe dehydration in paediatric patients [28]. Signs of dehydration include increased thirst, irritability, restlessness, lethargy, sunken eye, dry mouth and tongue, loss of skin tugor [28], and sunken fontanelle; decreased urine output, lack of interest in playing; drowsiness, tachypnea and tachycardia are also seen [28].

Rotavirus gastroenteritis leads to dehydration which may be mild or severe and life threatening. Milder cases have symptoms for short period (3 – 8days) only and recovery is complete [29]. In severe cases there is dehydration and severe loss of electrolytes which may result in life threatening complications such as hypovolemia, circulatory collapse and eventual death [28].

COMPLICATIONS:

Acute severe dehydration results in the following complications:

- Electrolyte imbalance - Hyponatremia and Hypokalemia leads to cardiac failure and seizures [42].
- Rapid loss of water and electrolytes which results in acidosis, shock and death[42]
- Aspiration of vomitus leading to pneumonitis is another complication linked with Rotavirus gastroenteritis
- It can cause chronic diarrhoea in immunocompromised children [29].

- It has been reported that frequent Rotavirus infection has been associated with Coeliac disease in genetically predisposed children [42].

DIAGNOSIS

Early diagnosis of Rotavirus gastroenteritis in hospitalized patients will decrease the morbidity and mortality considerably and avoids inappropriate use of antibiotics in paediatric patients.

It is difficult to differentiate Rotavirus gastroenteritis from other causes of gastroenteritis on clinical grounds alone. It may be necessary to establish Rotavirus as the etiological agent in some clinical conditions such as protracted diarrhoea, complicated cases, immunocompromised hosts and for epidemiological studies

DIAGNOSTIC METHODS:

***Rotavirus Can Be Detected From Stool Specimen By The Following Techniques*:**

Acute Rotavirus gastroenteritis was diagnosed by the conventional methods such as Electron-microscopy, Counter-immunoelectrophoresis, Reverse passive Haemagglutination assay (RPHA), DNA Oligonucleotide micro assay methods, and Tissue culture, Flowcytometry, Complement fixation test and Latex agglutination test(LAT). Subsequently, development of newer methods such as Polyacrylamide gelelectrophoresis (PAGE), Dot

hybridization technique, Immunoflourescence test, Enzymelinked immunospot assay, Immunochromatography (ICT), Enzyme immuno assay (EIA) and RT-PCR have replaced the conventional methods since, they are rapid, accurate and also having good sensitivity and specificity[2,14,26,29,40,45,47]

MANAGEMENT

- Isolation of the patient and prompt treatment
- Since Rotavirus is stable in room temperature and highly contagious isolation of the patient is necessary to prevent person to person transmission of the virus in the ward and the community [1]; the bed clothes and linens are decontaminated with chlorine or phenol
- Treatment of Rotavirus gastroenteritis is mainly by supportive measures which include prompt correction of water and electrolyte loss [29]; replacement of fluids and correction of electrolyte imbalance either by intravenous route or orally as feasible
- O.R.T (Oral Rehydration Therapy) was the single most effective strategy followed to prevent dehydration related mortality earlier [48].
- But there is risk of hypernatremia in standard ORS when given to children with non-cholera diarrhoea. Hence low osmolarity ORS is recommended by WHO and IAP (Indian Academy of Paediatrics) as

an universal solution for treatment and prevention of dehydration for all cases of diarrhoea and at all ages .

- In 2004, the WHO and UNICEF recommended low-osmolarity oral rehydration solution (ORS) and zinc supplementation for the treatment of acute diarrohea [49].
- Energy dense feeds in addition to exclusive breast feeding has been included in treatment of gastroenteritis by Ministry of Healthand Family Welfare, Government of India.
- Zinc supplementation: 10mg of (elemental zinc) given for a period of 2weeks for children in the age group of 2-6 months and 20 mg per day for children aged more than 6 months is being advised [49,50].
- Zinc has been linked with decreased illness and mortality by lowering the severity as well as duration of diarrhoea; it also lowers the incidence (UNICEF-PHFI Indian Paediatr-2012 PUBMED).

RISK FACTORS

- lack of sanitation, poor access to safe drinking water, lack of knowledge about hygienic measures contributes around 88% of diarrhoeal cases in children [51].

- In addition, low socioeconomic status, illiteracy, low birth weight, inadequate breast feeding, malnutrition are linked with higher incidence of diarrhoea in children[51]

PREVENTIVE MEASURES

- As the main mode of transmission of Rotavirus is through faeco-oral route, proper disposal of excreta, hand hygiene, use of safe drinking water, immunization against measles and promotion of exclusive breast feeding are the most important measures to prevent diarrhoeal diseases[49]
- Effective hand hygiene includes hand washing after defecation and before preparing food/ feeding their children
- Hand washing with soap and water can prevent the risk of diarrhoea by 42-47% (Lancet infect dis.,2003 PUBMED)
- In India, hand washing with soap before handling or consumption of food and after passing motion, is being encouraged in school children through the school hygiene program and mass media campaign on the **"hand washing day"** [49]; likewise, The World Toilet Day is conducted in the sense of giving awareness to the public to avoid open air defecation and its ill-effects[49]

- In addition, **vitamin A supplementation** is being promoted in children since it reduces the incidence of diarrhoea and associated deaths in children aged six months to five years[49]
- Furthermore, a safe and effective vaccine remains the promising way of reducing the worldwide burden of Rotavirus disease [5].

ROTAVIRUS VACCINES

Improvements in sanitation have only limited effect on Rotavirus occurrence. So it is important to introduce safe and effective immunization to decrease the impact of rotavirus infection in the community (Fischer et al.,).

The first Rotavirus vaccine – '**Rota-shield'** was introduced in United States (1998) by Wyeth and approved by USFDA (Food and drug administration) and ACIP (advisory committee on immunity practices); Clinical trials in the United States, Finland and Venezuela had proved the vaccine to be 80-100% effective in preventing severe gastroenteritis caused by group A Rotavirus [52,53]. However the vaccine was withdrawn from the market by the manufacturer in 1999 since it was associated with increased **risk of intussusception** (**1 in 12000 vaccinated infants)** among vaccinated children [54].

Two new vaccines (**Rotarix and Rota-teq**) against Rotavirus Group A infection were introduced in 2006 [54]. **Mexico** was the first among all countries in the world to introduce **Rotavirus vaccine** and the results showed

that **diarrhoeal deaths decreased by more than 65% in children less than 2 years.**

'Rotarix' is an oral live attenuated **monovalent vaccine**; the breadth of coverage depends on cross protection between serotypes. **'Rota-Teg'** is a **multivalent** modified Jenner vaccine. Bovine G serotypes combined with human P serotypes was found to be highly efficacious among severe rotavirus gastroenteritis caused by **G1or G2 serotypes** of Rotavirus. It was licensed in United States and European Union in 2006.

In 2009, the WHO recommended that Rotavirus vaccine can be included in all national immunization programs [56]. The two Rotavirus vaccines (Rotarix and Rota-teq) have been licensed in more than 100 countries, including India [57]. The incidences and severity of Rotavirus infection has declined dramatically in countries that have adopted this recommendation.

A median of 36% of children less than five years with acute diarrhoea showed positivity for Rotavirus based on the data from the Global Surveillance Network of 2009. Therefore, **WHO** has recommended inclusion of **Rotavirus vaccination** in all national immunization programs.

A Cochrane review (2012) of 41 clinical trials concluded Rotarix (GlaxoSmithline) and Rota-Teq (merk) were the effective vaccines and [58];

also reported that they were not associated with risk of intussception in immunized children.

Intense debate in introduction of Rotavirus vaccine in India was due to lack of estimates regarding mortality by Rotavirus diarrhoea and also expenditure of the vaccines.

A study indicates, 90,000 - 1,53,000 children die from Rotavirus infection each year in India; around 4% of overall mortality in children under five years could be saved in India, if Rotavirus vaccines were integrated with National Immunization Program [Shaun K Morris et al] (Bulletin of the World Health Organization 2012;90:720-727) [59].

In May 2013, India has developed a new vaccine against Rotavirus- '**Rotavac**'. The phase 3 clinical trials of low cost Indian-made Rotavirus vaccine-Rotavac have showed effective efficacy and excellent safety profile. It would be available at Rs.54 per dose if approved by the Drugs Controller General Of India [60].

It is found that the Rotavac reduced severe diarrhoea by more than 56% during first year of life with protection continuing into the second year also. Moreover, the vaccine also exhibited great impacts against severe diarrohea of any etiology [5].

Strains 116E and 1321 were isolated from NICU in New Delhi and Bangalore and pursued its vaccine property; A neonatal strain RV3 isolated by workers at Royal children hospital in Melbourne, Australia is being pursued as a vaccine character; bovine re-assortment vaccine was developed at NIH [41].

International non-governmental organization PATH, the WHO, the US, Centers for Disease Control Prevention, and the GAVI Alliance are working to bring Rotavirus vaccine to developing world, where the mortality and morbidity of diarrheoa in children are significantly high [5].

Integration of Rotavirus vaccination with National Immunization Program is advised in countries where diarrhoea associated mortality is more than 10% in children under five years [62]

Indian Council of Medical Research (ICMR) has formed a project called 'National Hospital Based Rotavirus Surveillance Network' to establish different surveillance units in tertiary care hospitals in different parts of India [5].

Objectives Of The National Hospital Based Rotavirus Surveillance Network: [5]

- To identify the proportion of diarrhoea and mortality attributable to Rotavirus infection in children less than 5 years of age

- To emphasis hospital based and community based study of Rotavirus infection in children
- To identify the susceptible age, seasonal distribution, characterization of strains

  (G and P types) in different geographical areas
- To isolate newly emerging strains or unusual strains attributed to zoonotic diseases or strains not identified by standard techniques
- To find out children admitted due to intussception and its causal relationship with Rotavirus vaccination if any[[61].

MATERIALS & METHODS

MATERIALS AND METHODS

Study area

This prospective study was conducted at the Department of Microbiology, Coimbatore Medical College, Coimbatore for a period of one year from July 2015 to June 2016.

Study population

Overall, 100 children less than five years with acute gastroenteritis from outpatient department and ward were included in the study population.

Inclusion criteria

- Children under five years with acute gastroenteritis
- Children below five years with diarrhoea alone

Exclusion criteria

- Children under five years with bloody diarrhoea
- Children less than five years who acquired diarrhoea during hospitalization (Healthcare Associated Infection/Nosocomial infection)
- Children below five years with chronic diarrhoea (diarrhoea >14 days)
- Children under five years with immuno compromised state

Ethical clearance

Ethical committee approval was obtained from the institutional review board of Coimbatore Medical College, Coimbatore. Detail about the research was informed to the parents/guardians of the participant; oral as well as written consent were obtained for participating in the study.

Clinical history

Detailed history was taken from the parents/guardian of the patient and entered in the proforma as follows:

- Name, age, gender, geographical area (urban/rural), occupation, literacy and socioeconomic status
- Antenatal history- full term/preterm, birth weight, immunization
- Onset and duration of diarrhoea, whether blood stained or not, vomiting, abdominal pain, and fever
- Feeds- breast fed/bottle fed
- Hand washing and other sanitary measures
- Physical examination of the child to detect signs of dehydration- consciousness, whether the baby taking feeds, active, skin tugor, anterior fontenelle, sunken eyes.
- Macroscopical examination of stool specimen – watery, colour, whether blood stained or not.

Sample collection, transport and storage

- About 15-20 ml of stool specimen was collected in a sterile wide mouthed universal container during acute stage of gastroenteritis (< 3 days of onset of symptoms)
- Samples were kept in ice box to maintain ideal temperature (2-8^0 C) and transported immediately to the Microbiology diagnostic laboratory. The stool was incorporated into 2 ml sterile centrifuge container along with buffer and stored at -20 to -70^{0}C for detection of Rotavirus antigen.

Testing

The specimens were screened for Rotavirus antigen through Enzyme Linked Immuno Sorbent Assay (ELISA) and Immuno Chromatography Test (ICT) and confirmed by Reverse Transcriptase Polymerase Chain Reaction (RT-PCR).

Immuno Chromatography (ICG)

To detect the Rotavirus antigen from stool specimen, SD Bioline Rota rapid test kit, (Standard Diagnostics, Korea) was used. The kit works on the principle of Immuno chromatography. The specimens were processed as per the kit manufacturer's instructions.

Principle:

- This test uses two types of antibody in a solid phase, i.e., sandwich immuno chromatography for detection of group specific antigen which includes the major inner capsid protein of Rotavirus group A.
- Nitrocellulose membrane precoated with rabbit monoclonal anti Rotavirus antibodies
- Mouse monoclonal anti Rotavirus antibodies were used as detector agents
- The mouse monoclonal anti Rotavirus antibody-colloid reacts specifically with G-anti Rotavirus antibody in human stool specimen ; this mixture reacts with the rabbit anti Rotavirus antibody present in the nitrocellulose membrane
- The cassette has two lines C-control and T-test on the device.
- For the test to be valid, the control line should always appear.

Contents of the kit:

1. Foil pouch with desiccant
2. Sample collection tube
3. Assay diluents
4. Sample collection swab
5. Disposable dropper

PROCEDURE:

Preparation of extracted sample

- Test device and samples are allowed to come to room temperature
- Assay diluent was taken in the given disposable dropper up to the line shown on it; then transferred into the sample collection tube; this step is repeated once again
- About 50 mg of stool sample was taken with sample collection swab
- The swab is then inserted into the sample collection tube
- Swab is swirled (up to 10 times) until the sample has been dissolved into the assay diluent and the swab squeezed against the tube wall, then discarded

Test procedure:

- The test device was removed from foil pouch and placed on a flat, dry and clean surface
- Dropping cap was assembled on the sample collection tube
- 4-5 drops of sample was added to sample well of the test device
- Purple colour appears across the result window in the Centre of the test device which indicates the test is working
- The result was interpreted at 10-20 minutes

Interpretation:

- A control band, purple in colour will appear in the left section of the result window. This shows that the test kit is working properly
- The right section of the result window indicates the test result

Negative result:

The presence of only the control band and not the test band within the result window indicates a negative result

Positive result:

The presence of two colour bands on the result window, test band (T) and control band (C) indicates a positive result, no matter whichever band appears first

ELISA (Enzyme Linked Immunosorbent Assay)

This test is done for detection of Rotavirus antigen from human stool specimen; it is a sensitive method and can be used to detect the rise in antibody titer [29].

Principle:

- It is a solid phase sandwich type Enzyme Immuno Assay (EIA)
- The wells of the microtitre plate were coated with monoclonal antibody against the group specific antigen for all known human Rotaviruses

- An aliquot of stool specimen is added and anti Rotaviral monoclonal antibody conjugated to horse radish peroxidase are added simultaneously into the well and incubated for sixty minutes at room temperature
- The Rotaviral antigen is being caught between the solid phase and enzyme linked antibodies
- Incubated for 60 minutes, well is washed to remove unbound enzyme linked antibodies
- Enzyme substrate A (urea peroxidase) and substrate B (tetra methyl benzaldehyde) are added into the well and incubated for a period of ten minutes at room temperature
- The enzyme bound in the well converts the colourless substrate to blue colour; the intensity of blue colour is directly proportional to the concentrate of Rotaviral antigen in the stool specimen

Contents:

- Microtitre well, coated with Rotaviral monoclonal antibody
- Conjugate (horse radish peroxidase conjugated to anti Rotaviral monoclonal antibody)
- Positive control (Inactivated simian Rotaviral-SA-11)
- Sample diluents
- Part-A substrate buffer

- Part-B substrate solution
- Stop solution
- Sample transfer pipettes
- Microtitre well holder

Specimen preparation:

- One ml of sample diluent was added in a sterile test tube with a micro pipette and sample was added to the diluent through transfer pipette

Test procedure:

- Adequate number of wells were snapped off including controls and inserted into the microtitre well holder
- Sample position was labeled and recorded
- 100 µl of diluted stool sample, positive control and negative control (SD) were added to the bottom of separate wells
- 100 µl enzyme conjugate was added to each well; contents in the well were mixed thoroughly by gentle swirling on table top
- Wells were incubated at room temperature for 60 minutes
- Liquid was poured out of the wells into the decontamination jar; then the microtitre well holder was tapped upside down vigorously against an absorbent paper to remove fluid completely from the wells

- And the wells were filled with distilled water and the liquid was poured out as in the previous step and repeated four more times, totally 6 times.
- 100 µl of substrate A solution and 100ml of substrate B solution were added into each wells
- Wells were incubated at room temperatures for 10 minutes
- 100 µl stop solution was added to each well
- Reading was taken within 60 min of adding stop solution using the ELISA reader.

Interpretation:

Results can be determined visually or by spectrophotometry.

Positive result by visual determination:

Samples which showed blue colour more intense than that of the negative control were taken as positive; samples of equal colour or less intense than that of the negative control is taken as negative

Positive result by spectrophotometric method:

After adding stop solution, absorbance value for every well was read at 450 nm.

Specimen with absorbance unit (A 450) >0.150 were taken as positive; Specimen with absorbance value equal or <0.150 were taken as negative.

Real –Time PCR detection of Rotavirus-A

Material and Methods:

PureFast ^{R} Viral nucleic acid mini spin purification kit. The kit contains Proteinase-K, Lysis buffer, Wash buffer-1, Wash buffer-2, Spin columns with collection tube and elution buffer. HELINI Rotavirus-A Real-time PCR kit is from HELINI Biomolecules, Chennai, India.

Real Time PCR model: Agilent MX3000P, USA

HELINI Rotavirus-A Real-time PCR kit components:

1. RT-PCR Probe PCR Master Mix
2. RT-Enzyme Mix
3. Rotavirus-A Primer Probe Mix
4. Internal Control Primer Probe Mix
5. Internal Control Template
6. Positive Control
7. Instruction Manual

Viral RNA Purification

1. 100 mg of faecal sample is mixed with 2 ml of stool processing buffer
2. Vortexed well and centrifuged @ 10000 rpm for 10 min
3. Clear supernatant transferred into fresh 1.5 ml centrifuge tube
4. 400 µl of supernatant transferred into fresh 1.5 ml centrifuge tube and equal volume (400 µl) of lysis buffer added
5. Mixed well by vortex
6. Add 20 µl of Proteinase K and 5 µl of internal control template, mixed well by inverting several times
7. Incubated at 56 deg C for 15 min
8. Add 400 µl of Ethanol and mixed well
9. Transferred entire sample into the Pure fast
10. Added 500µl Wash buffer-1 to the Purefast ^{R} spin column. Centrifuged at 10000rpm for 1 min and discarded the flow-through. Placed the column back into the same collection tube.
11. Added 500 µl Wash buffer-2 to the purefast ^{R} spin column, Centrifuged at 10000rpm for 1 min and discard the flow-through. Place the column back into the same collection tube.
12. Discarded the flow-through and centrifuged for one more 1 min.This step is essential to avoid residual ethanol.

13. Transferred the Purefast^{R} spin column into a fresh 1.5ml micro-centrifuge tube.
14. Added 100ml of Elution Buffer to the Purefast^{R} spin column membrane.
15. Incubated for 1 min at room temperature and centrifuged for 2 min.
16. Discarded the column and stored the purified DNA at -20^{0} C. 10µl of elute used in real-time PCR analysis.

PCR procedure:

Detection mix

Components	Volume
RT-Probe PCR Master Mix	8µl
RT enzyme mix	2µl
Rotavirus-A Primer Probe Mix	2.5µl
Internal control Primer Probe Mix [IC PP Mix]	2.5µl
Purified Viral RNA sample	10µl
Total Reaction Volume	25µl

PCR vials Centrifuged briefly before placing into thermal cycler.

Negative Control Setup

Included 10µl of nuclease free water as negative control.

Positive Control Setup

Included 10µl of positive control.

Amplification Protocol

	Step	**Time**	**Temp**
	Reverse transcriptase	30min	42º C
	Taq enzyme activation	15min	95º C
50 Cycles	Denaturation	20sec	95º C
	Annealing/Data collection*	20sec	56º C
	Extension	20sec	72º C

Rotavirus-A = FAM channel

Internal Control = JOE/HEX/VIC/Cy3 Channel

Advantage:

RT-PCR is the most sensitive method for detection of Rotavirus antigen from stool specimen [29].

Disadvantage:

Though it is rapid and accurate method for detection of Rotavirus antigen from stool specimen, it is not cost effective; so routinely not used in the diagnosis of Rotavirus gastroenteritis.

Rotavirus- A Genotyping

Material & Methods:

Purefast Viral DNA purification kit, PCR Master Mix, Agarose gel electrophoresis consumables and HELINI Rotavirus-A Genotyping PCR kit are from HELINI Biomolecules, Chennai, India.

2X Master Mix:

It contains 2U of Taq DNA polymerase, 10X Taq reaction buffer, 2mM MgCl2, 1µl of 10mM dNTPs mix and PCR additives.

Agarose gel electrophoresis:

Agarose, 50X TAE buffer, 6X gel loading buffer and Ethidium bromide are from HELINI Biomolecules, Chennai.

Rotavirus-A Genotypes - G

Set-1

G1 – 215bp

G2 – 351bp

G3 – 451bp

G4 – 582bp

G8 – 676bp

Set-2

G9 – 387bp

G10 – 250bp

G12 – 550bp

Rotavirus-A Genotypes - P

P4 – 369bp

P6 – 580bp

P8 – 250bp

P9 – 287bp

P10 – 398bp

P11 – 563bp

Genotyping cDNA Protocol

Three tubes for each sample [Sample-1, 3, 7, 8, 9 and 13]

Components	**Genotype-G set-I**	**Genotype-G set-II**	**Genotype-P**
cDNA reaction Mix	**6µl**	**6µl**	**6µl**
RT-Enzyme mix	**2µl**	**2µl**	**2µl**
Genotype Primer Mix	**4µl**	**4µl**	**4µl**
Purified Viral RNA	**8µl**	**8µl**	**8µl**
Total reaction volume	**20µl**	**20µl**	**20µl**

Centrifuge PCR vials briefly before placing into thermal cycler.

Incubated at 42°C for 1hour.

PCR Protocol

Three tubes for each sample [Sample-1, 3, 7, 8, 9 and 13]

Components	**Genotype-G set-I**	**Genotype-G set-II**	**Genotype-P**
RedDye PCR Master Mix	**10µl**	**10µl**	**10µl**
Genotype Primer Mix	**4µl**	**4µl**	**4µl**
Water	**9µl**	**9µl**	**9µl**
cDNA	**2µl**	**2µl**	**2µl**
Total reaction volume	**25µl**	**25µl**	**25µl**

Centrifuge PCR vials briefly before placing into thermal cycler.

Amplification Protocol

	Step	**Time**	**Temp**
	Taq enzyme activation	5min	95ºC
35 Cycles	Denaturation	30sec	95ºC
	Annealing	30sec	58ºC
	Extension	45sec	72ºC
	Final extension	5min	72ºC

Gel electrophoresis:

Prepare 2% agarose gel as per standard procedure. Loaded entire PCR amplified product along with 10µl of 100bp DNA Ladder. Run electrophoresis and visualize in UV Transilluminator.

Agarose gel electrophoresis:

1. Agarose (2%) prepared by adding 2 gms agarose in 100ml of IX TAE buffer and melted by using micro oven.
2. Added 5µl of Ethidium bromide when the agarose gel temperature reaches around 60^{o}C.
3. Warm agarose solution poured slowly into the gel platform.
4. Kept the gel set undisturbed till the agarose solidifies.
5. IX TAE buffer poured into submarine gel tank.
6. The gel platform was carefully placed into tank and the tank buffer level 0.5cm above than the gel was maintained.
7. PCR Samples are loaded after mixed with gel loading dye along with 10µl HELINI 100bp DNA Ladder.
8. Run electrophoresis at 50V till the dye reaches three fourth distance of the gel.
9. Gel viewed in UV Transilluminator and observed the bands pattern.

FIG 1: IMMUNOCHROMATOGRAPHY KIT WITH ITS CONTENTS

(SD Rotavirus)

Fig 2: IMMUNOCHROMATOGRAPHY CASSETTES
(i) Negative Sample and (ii) Positive Sample

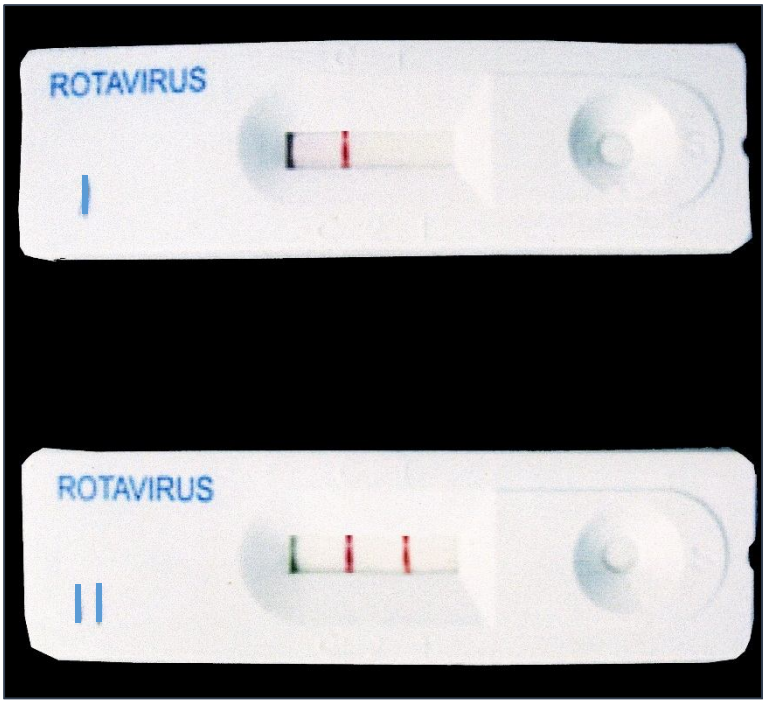


Fig 3: ELISA KIT AND ITS CONTENTS

Fig 4: STOOL SAMPLES AND MICROTITRE PLATE

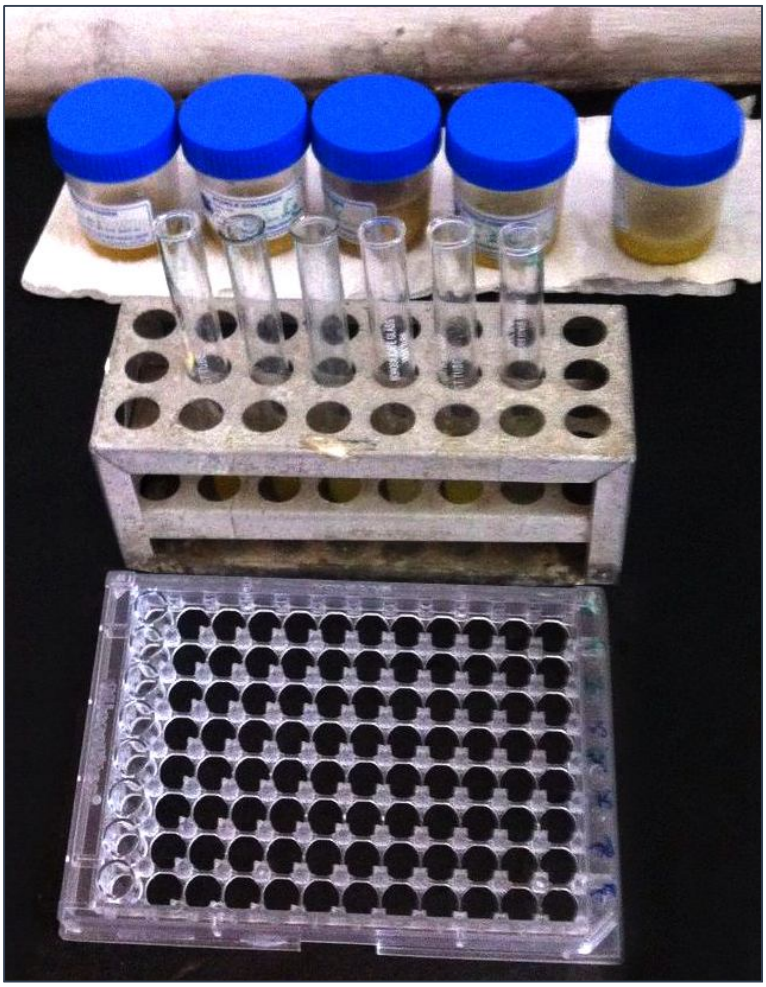


Fig 5:ELISA READER

Fig 6: ELISA MICROTITRE PLATE WITH CONTROLS AND SAMPLES

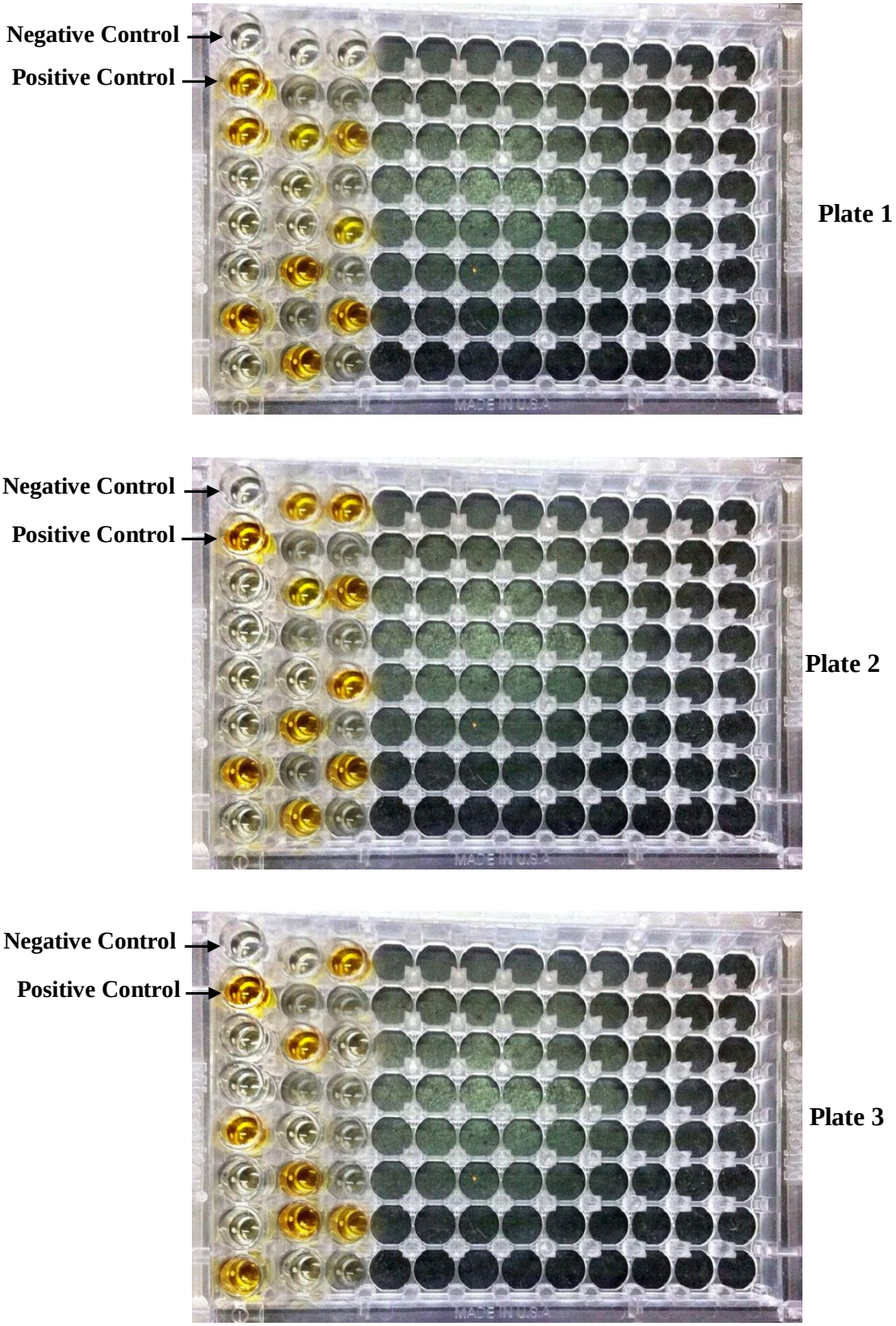
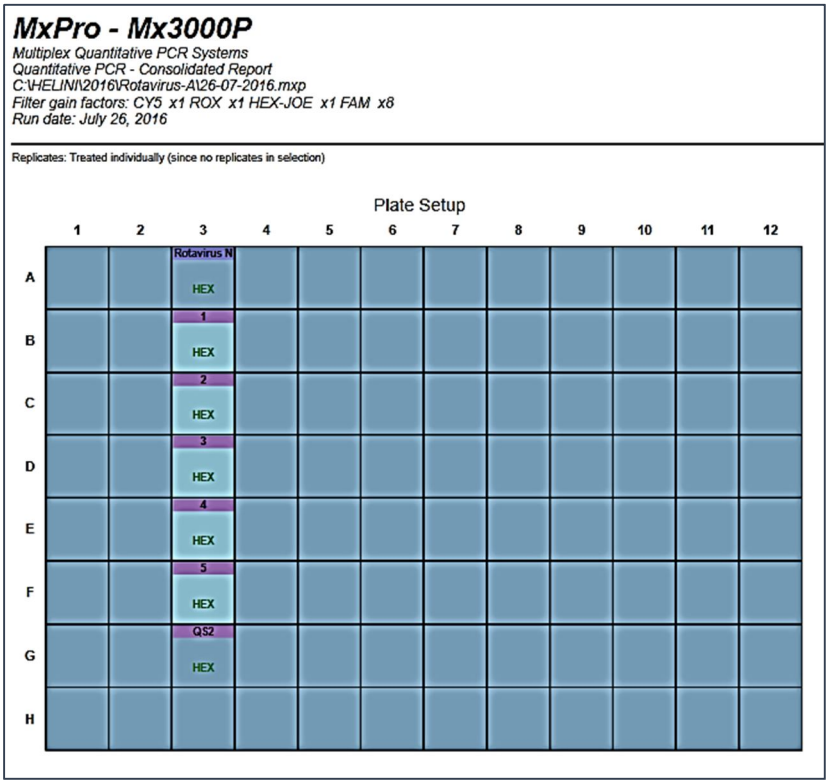


Fig 7a: RT - PCR ROTAVIRUS: PLATE SETUP AND THERMAL PROFILE



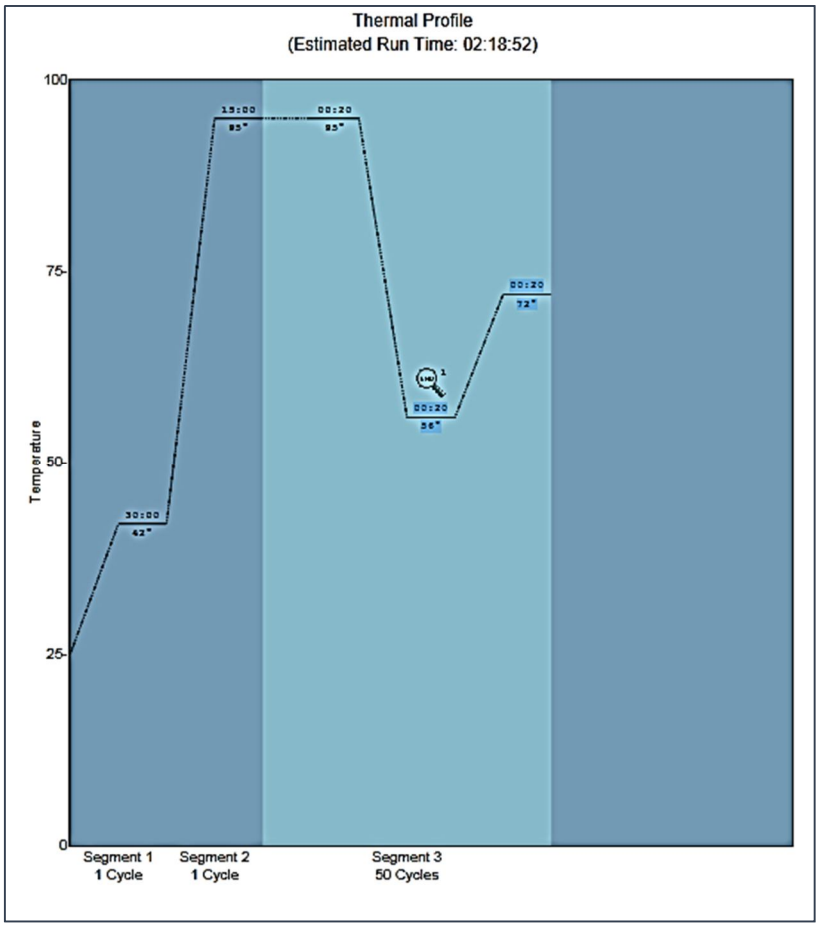
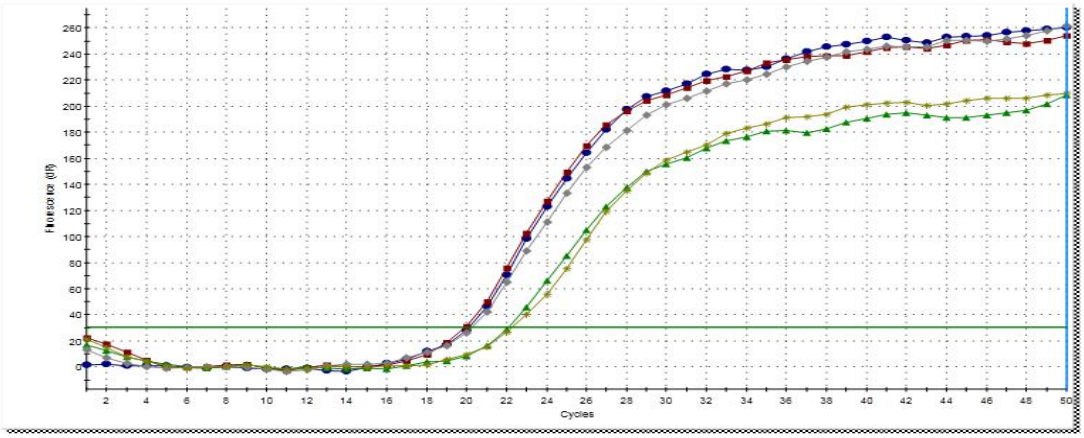


Fig 7b: RT PCR GROUP A ROTAVIRUS AMPLIFICATION PLOTS

MxPro - Mx3000P

Multiplex Quantitative PCR Systems
Quantitative PCR - Consolidated Report
C:\HELINI\2016\Rotavirus-A\26-07-2016.mxp
Filter gain factors: CY5 x1 ROX x1 HEX-JOE x1 FAM x8
Run date: July 26, 2016

Amplification Plots



MxPro - Mx3000P

Multiplex Quantitative PCR Systems
Quantitative PCR - Text report
C:\HELINI\2016\Rotavirus-A\26-07-2016.mxp
Filter gain factors: CY5 x1 ROX x1 HEX-JOE x1 FAM x8
Run date: July 26, 2016

Thermal Profile Summary

Segment	Cycles	Plateau	Temp. (degrees)	Temp. Inc. (deg/sec)	Duration (min:sec)	Time Inc. (min:sec)	Collect
1	1	Plateau 1	42.0	0.0	30:00	00:00	<none>
2	1	Plateau 1	95.0	0.0	15:00	00:00	<none>
3	50	Plateau 1	95.0	0.0	00:20	00:00	<none>
3	50	Plateau 2	56.0	0.0	00:20	00:00	1 Endpoints
3	50	Plateau 3	72.0	0.0	00:20	00:00	<none>

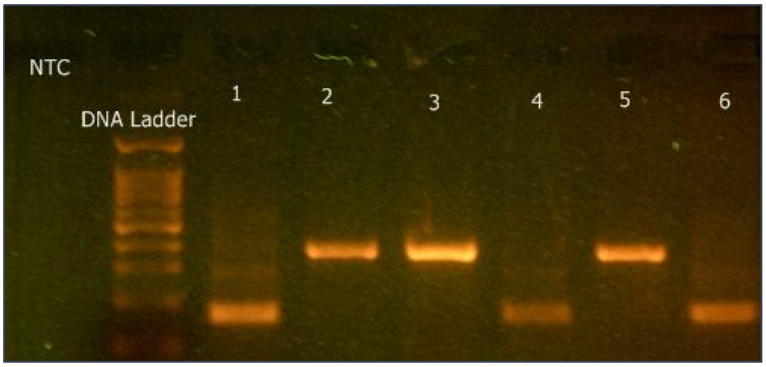
Replicates: Treated individually (since no replicates in selection)
* Fluorescence term used: dR

Text Report

Well	Well Name	We	Dye	Well Type	Threshold*	Ct*
B3	1	---	HEX	Unknown	30.155	20.14
C3	2	---	HEX	Unknown	30.155	19.94
D3	3	---	HEX	Unknown	30.155	22.11
E3	4	---	HEX	Unknown	30.155	20.29
F3	5	---	HEX	Unknown	30.155	22.31

Fig 7c: GROUP A ROTAVIRUS GENOTYPING

P - GENOTYPING



G - GENOTYPING

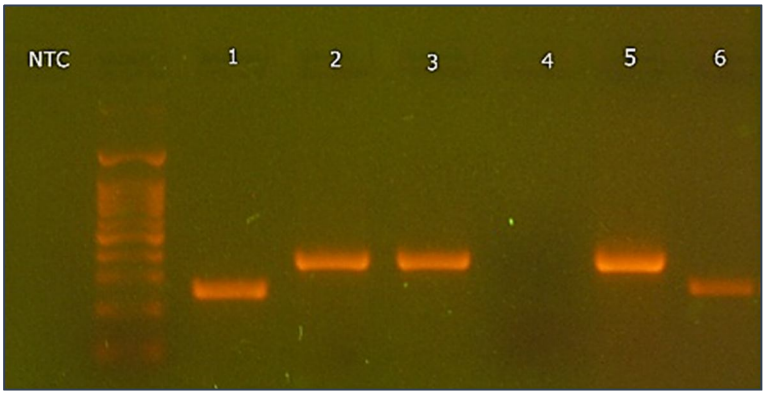
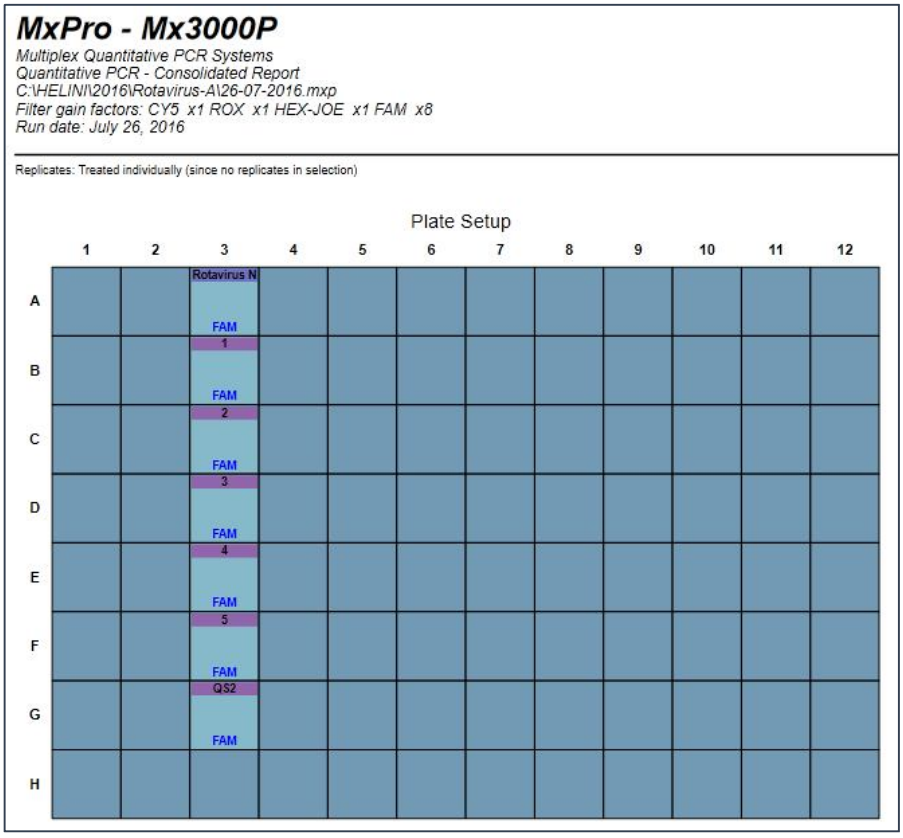


Fig 8a: RT - PCR ROTAVIRUS: PLATE SETUP & THERMAL PROFILE



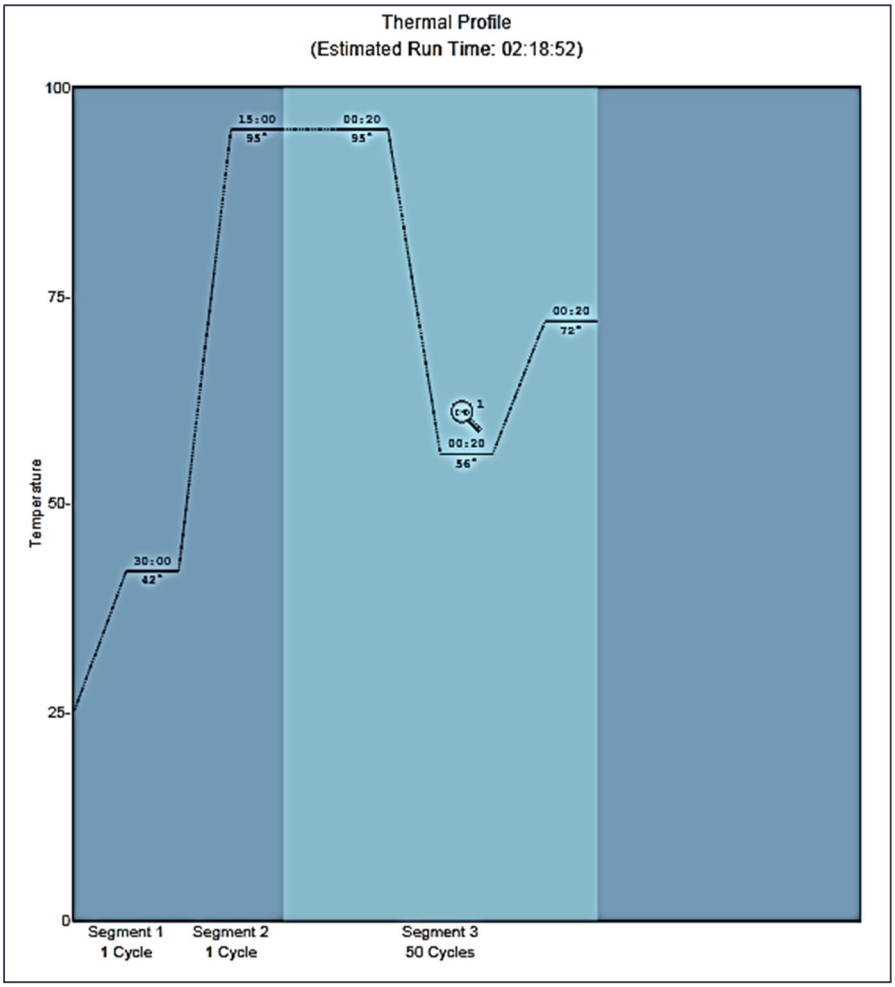


Fig 8b: RT PCR GROUP A ROTAVIRUS AMPLIFICATION PLOTS

MxPro - Mx3000P

Multiplex Quantitative PCR Systems
Quantitative PCR - Consolidated Report
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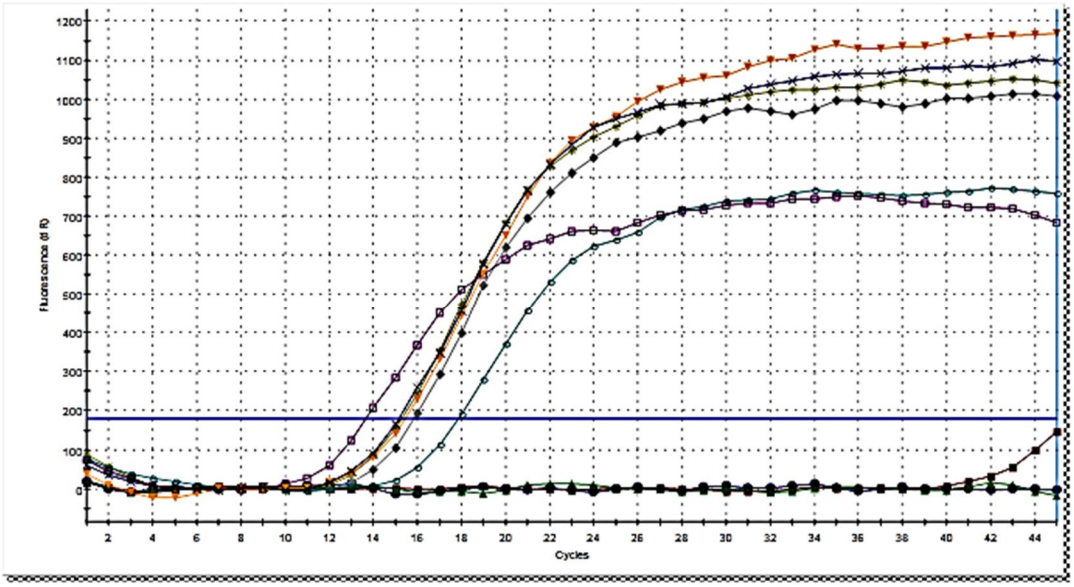
Filter gain factors: CY5 x1 ROX x1 HEX-JOE x1 FAM x8
Run date: August 06, 2016

Replicates: Treated individually (since no replicates in selection)

Amplification Plots



MxPro - Mx3000P

Multiplex Quantitative PCR Systems
Quantitative PCR - Text report
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Filter gain factors: CY5 x1 ROX x1 HEX-JOE x1 FAM x8
Run date: August 06, 2016

Thermal Profile Summary

Segment	Cycles	Plateau	Temp. (degrees)	Temp. Inc. (deg/sec)	Duration (min:sec)	Time Inc. (min:sec)	Collect
1	1	Platesu 1	95.0	0.0	15:00	00:00	<none>
2	45	Platesu 1	95.0	0.0	00:20	00:00	<none>
2	45	Platesu 2	56.0	0.0	00:20	00:00	1 Endpoints
2	45	Platesu 3	72.0	0.0	00:20	00:00	<none>

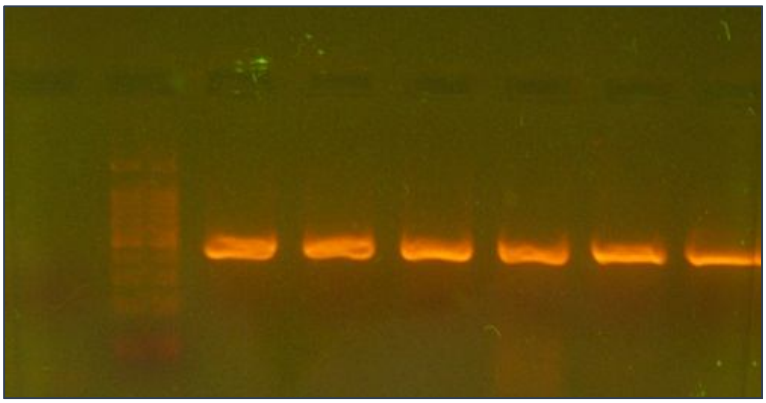
Replicates: Treated individually (since no replicates in selection)
* Fluorescence term used: dR

Text Report

Well	Well Name	Dye	Well Type	Threshold*	Ct*
A2	NTC	FAM	NTC	179.242	No Ct
A6	6	FAM	Unknown	179.242	No Ct
A10	7	FAM	Unknown	179.242	No Ct
B2	1	FAM	Unknown	179.242	15.88
C2	2	FAM	Unknown	179.242	15.33
D2	3	FAM	Unknown	179.242	17.89
D4	QS2	FAM	Unknown	179.242	13.72
E2	4	FAM	Unknown	179.242	15.50
F2	5	FAM	Unknown	179.242	15.17

Fig 8c: GROUP A ROTAVIRUS GENOTYPING

P - GENOTYPING



G - GENOTYPING

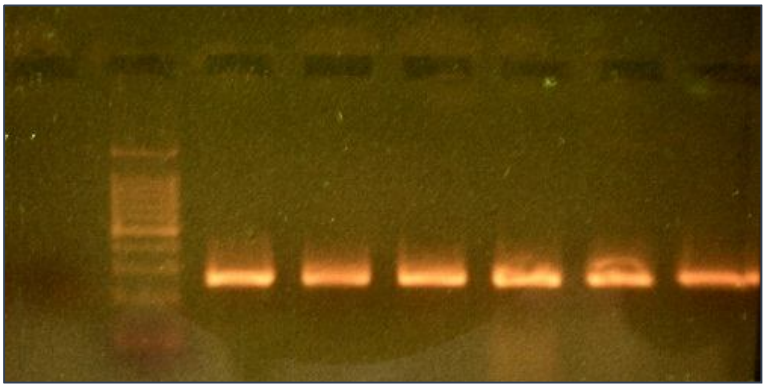


Fig 9 PCR MACHINE – Aligent MX3000P, USA

RESULTS

RESULTS

Prevalence of Rotavirus

During the study period of one year (July 2015 to June 2016), 100 children below five years with acute gastroenteritis were screened for Rotavirus antigen; of which, 56 (56%) were male and 44 (44%) were female. Out of 100 samples, 24 were positive for Rotavirus antigen.

Gender distribution of Rotavirus

In this study, out of 100 children 24 were positive for Rotavirus antigen; of which male children showed 58% (14/24) and female children showed 42% (10/24) positivity [Chart No. 2].

Age Distribution of Rotavirus

The maximum number of Rotavirus positivity was seen in the age group of 6 to 12 months (58.3), followed by 13 to 24 months (25%). The infection rate was found to be lower in the age group of 0 to 6 months (8.3)**;** likewise after 2 years of age, Rotavirus infection decreases sharply (8.3%) [Table No. 2] .

Seasonal Trends of Rotavirus

Even though the occurrence of Rotavirus was observed throughout the year, the maximum cases were found during the cooler months of the year (September to February) than other months [Chart No. 3].

Geographical Distribution of Rotavirus

Despite Rotavirus being prevalent in all geographical areas, 22% of cases were from urban whereas 28% from rural areas in this study [Chart No. 4 and Table No. 4].

Rotavirus infection related with water consumption

In this study higher incidence of Rotavirus positive cases were found in those using public water supply (25.3%) than those used bore well water(20.7%). It indicates there is a higher frequency of Rotavirus infection in those using public water supply than bore wells [Table No. 5].

Literacy of Parents

Poor literacy of parents is linked with increased rate of diarrhoeal diseases in young children (76) [Chart No. 5].

Socio Economic Status of Parents

In this study eighty percent of Rotavirus infected children belongs to low socioeconomic group and 20% from middle class It shows low socio

economic status has been linked with higher incidence of rotavirus gastroenteritis [Chart No. 6] .

Rotavirus Gastroenteritis Related with Feeds

In this study, lower incidence of gastroenteritis were seen among children who were exclusive breast fed (18%) whereas higher incidence of gastroenteritis were found in children who were on mixed fed (27%) in addition to breast fed [Table 6] .

Clinical Features

Vomiting was found in 83% of Rotavirus positive cases while it was 54% in Rotavirus negative cases. Severe dehydration was observed more consistently with Rotavirus positive patients (79%) whereas it was less in Rotavirus negative cases (24%). Fever was noted more or less equally in both Rotavirus positive and negative cases [Table 7].

Diagnosis

Out of 100 samples tested for Rotavirus antigen by ELISA and ICG method, 24 were positive and 76 were negative which indicates both methods are equally sensitive and specific [Table No. 8].

RT-PCR AND GENOTYPING:

Out of 24 Rotavirus positive samples (by Elisa method), 12 samples were sent to the Helini Biomolecules, Chennai for PT-PCR and VP7(G) and VP4(P) genotyping. Of which, 2 samples did not amplify which may be due to degradation of the RNA; remaining 10 samples were amplified and genotyped in accordance with G and P types. The results showed that 6 samples (60%) were belong to G1[P8] combination which is the most common genotype prevalent throughout the world [89]; three samples(30%) were belong to G2[P4] combination; one sample is un-typable [Table No. 9].

Table No. 1: Distribution of Acute Gastroenteritis In Accordance With Age and Gender

Age	Male	Female	Total
0-6 months	8(8%)	5(5%)	13(13%)
6-12 months	21(21%)	18(18%)	39(39%)
13-24 months	15(15%)	12(12%)	27(27%)
25-60 months	12(12)	9(9%)	21(21%)
Total (0-60 months)	**56(56%)**	**44(44%)**	**100(100%)**

Table No. 2: Age Distribution in RVGE Cases

Age	No. of Cases	No. of positive	No. of negative
0-6 months	13	2 (15.4%)	11(84.6%)
6-12 months	39	14 (36%)	25(64%)
13-24 months	27	6 (22.2%)	21(77.8%)
25-60onths	21	2 (9.5%)	19(90.5%)

Table No. 3: Seasonal Distribution of Rotavirus

Month	No. of AGE cases	No. of Rotavirus positive cases	No. of Rotavirus negative cases
July	4	1 (25%)	3 (75%)
August	5	1 (20%)	4 (80%)
September	7	2 (28.5%)	5 (71.5%)
October	8	2 (25%)	6 (75%)
November	12	3 (25%)	9 (75%)
December	15	4 (26.7%)	11 (73.3%)
January	17	4 (23.5%)	13 (76.5%)
February	9	2 (22.2%)	(77.8%)
March	5	1 (20%)	4 (80%)
April	6	1 (16.6%)	5 (83.4%)
May	5	1 (20%)	4 (80%)
June	7	2 (28.5%)	5 (71.5%)
Total	100	24	76

Table No. 4: Geographical Distribution of Rotavirus:

Geographical area	No. of cases	No. of Rotavirus positive cases	No. of Rotavirus negative cases
Urban	**60**	**13 (22%)**	**47 (78%)**
Rural	**40**	**11 (28%)**	**29 (72%)**
Total	**100**	**24**	

Table No. 5: RVGE and water consumption:

Water supply	No. of cases	No. of Rotavirus positive cases	No. of Rotavirus negative cases
Public water	71	18(25.3%)	53(74.6%)
Bore well water	29	6(20.7%)	23(79.3%)

Table No. 6: Rotavirus Gastroenteritis cases related with feeds:

Feeds	No. of cases	No. of Rotavirus positive cases	No. of Rotavirus negative cases
Breast feed	33	6 (18%)	27 (82%)
Mixed feed	67	18 (27%)	49 (73%)
Total	100	24	76

Table No. 7: Clinical findings in Rotavirus Positive and Negative cases

Clinical features	Rotavirus positive cases (%)	Rotavirus negative cases (%)
Vomiting	83	54
Diarrhoea	100	100
Dehydration: Severe Mild	 79 25	 21 75
Fever	88	83

Table No. 8: Comparison of ELISA and ICT in Detection of Rotavirus Antigen

Test	No. of specimens	Positive (%)	Negative (%)
Elisa	100	24	76
ICT	100	24	76

Table No. 9: Genotypic distribution of Rotavirus

Genotype	No of Samples
G1[P8]	6 (60%)
G2[P4]	3 (30%)
Un-typable	1 (10%)

Chart No: 1 Age and Sex Distribution in RVGE Cases.

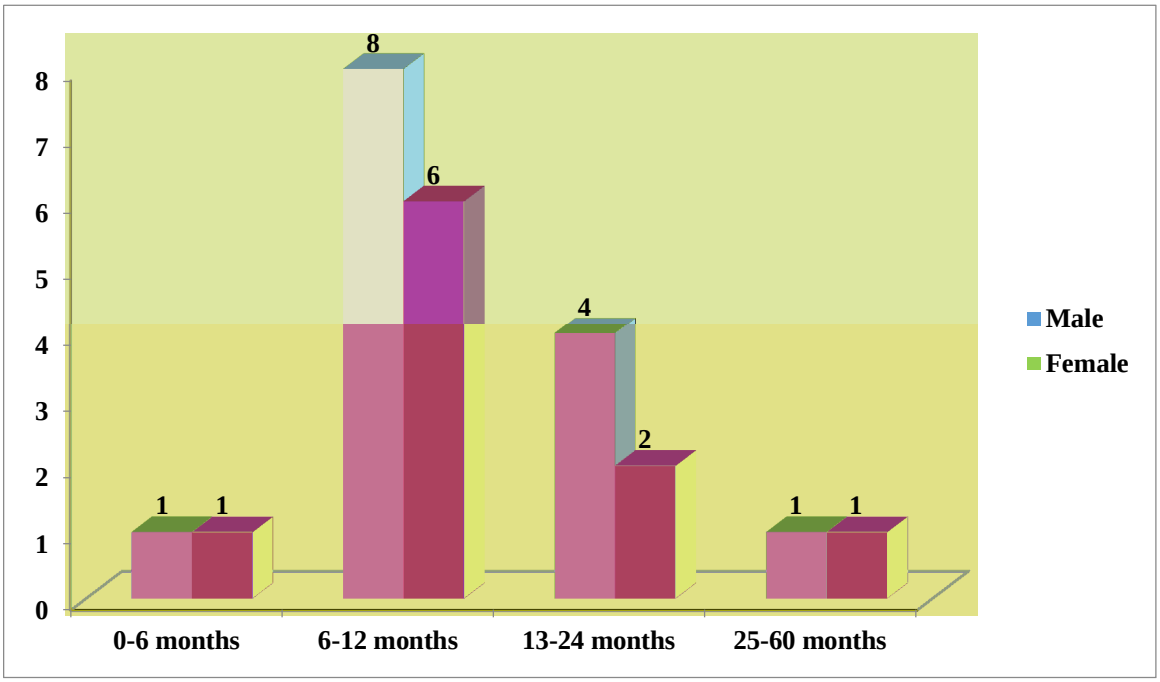


Chart No.2 Sex Distribution of study population and Rotavirus positive cases.

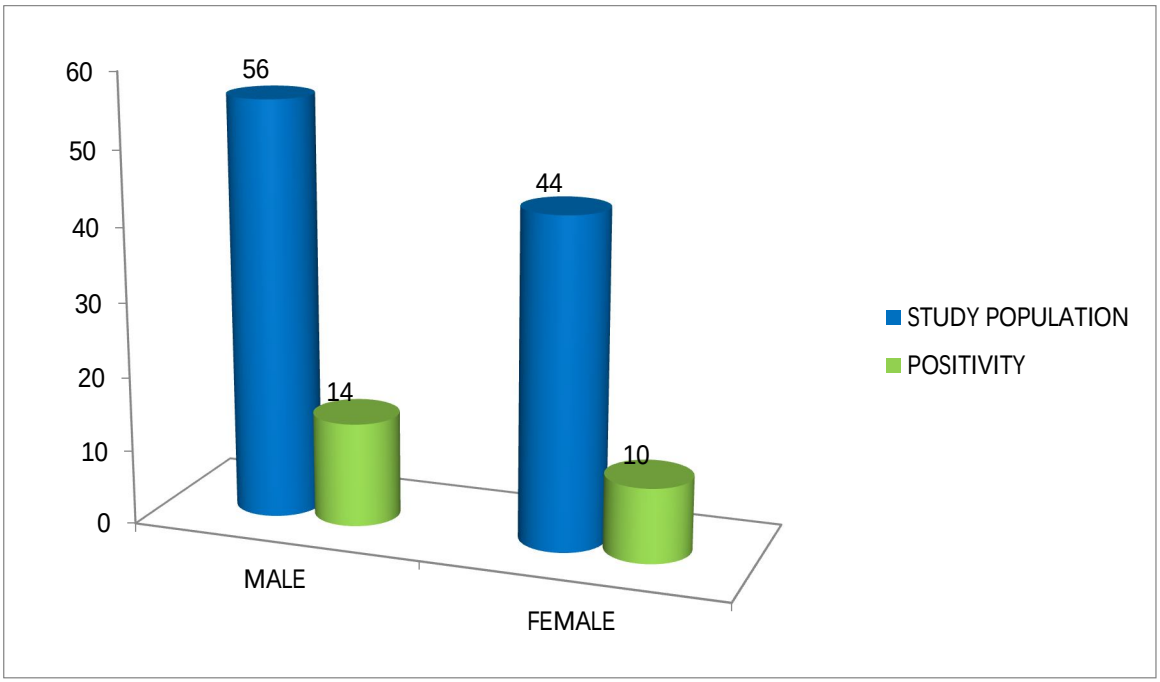


Chart No. 3 Seasonal Trends of Rotavirus.

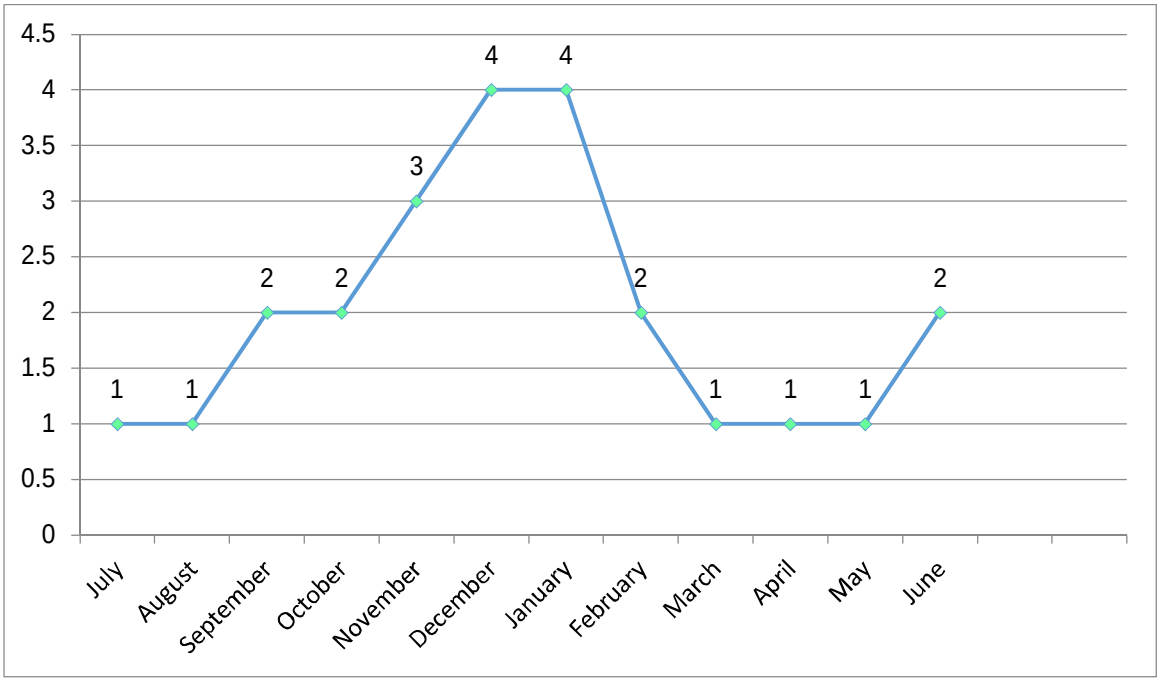


Chart No. 4 Geographical Distribution of Rotavirus.

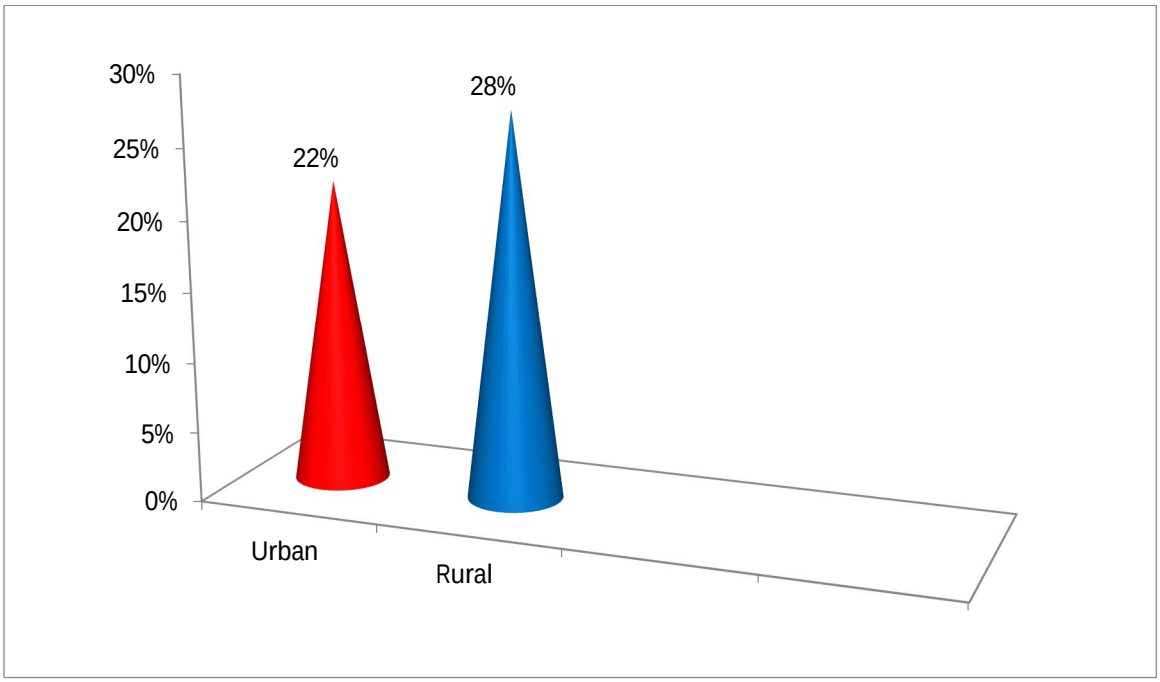


Chart No. 5 Educational Status of the Parents.

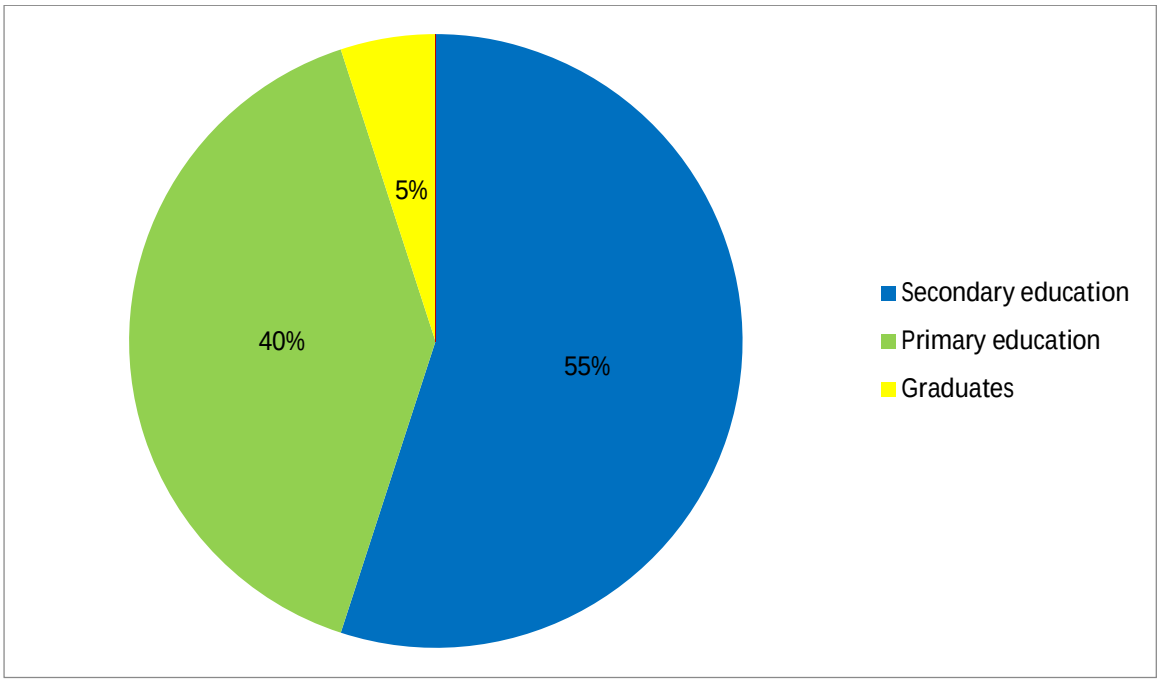


Chart No. 6 Socioeconomic Status of parents.

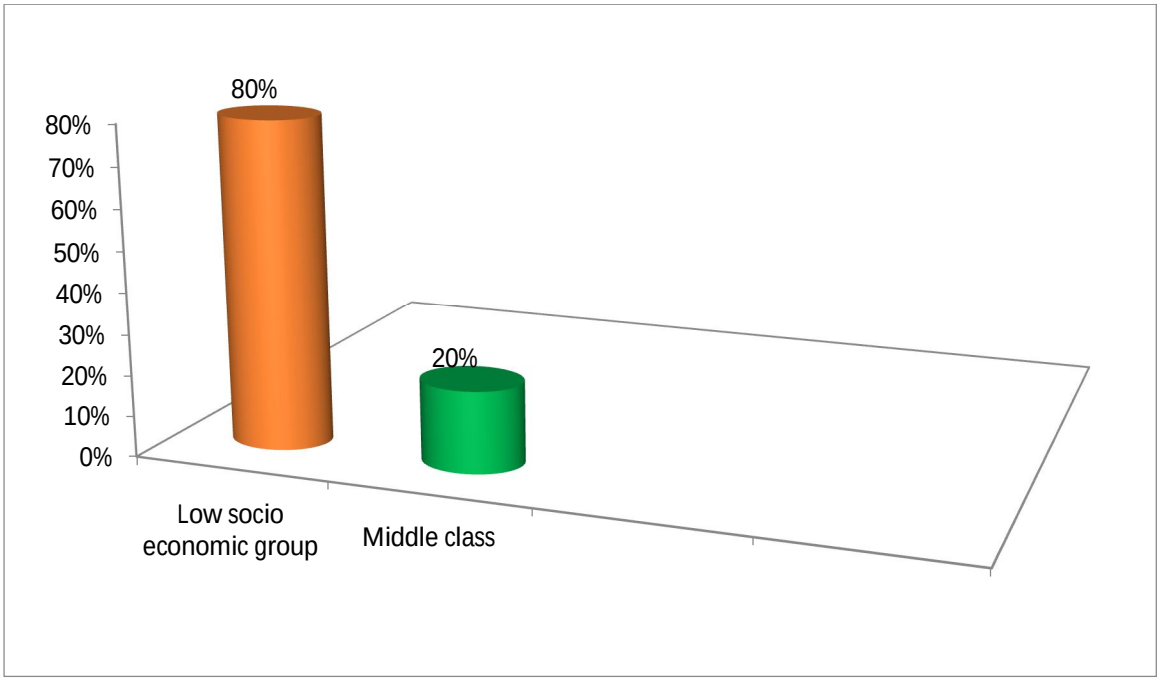


Chart No.7 Rotavirus Gastroenteritis and feeds.

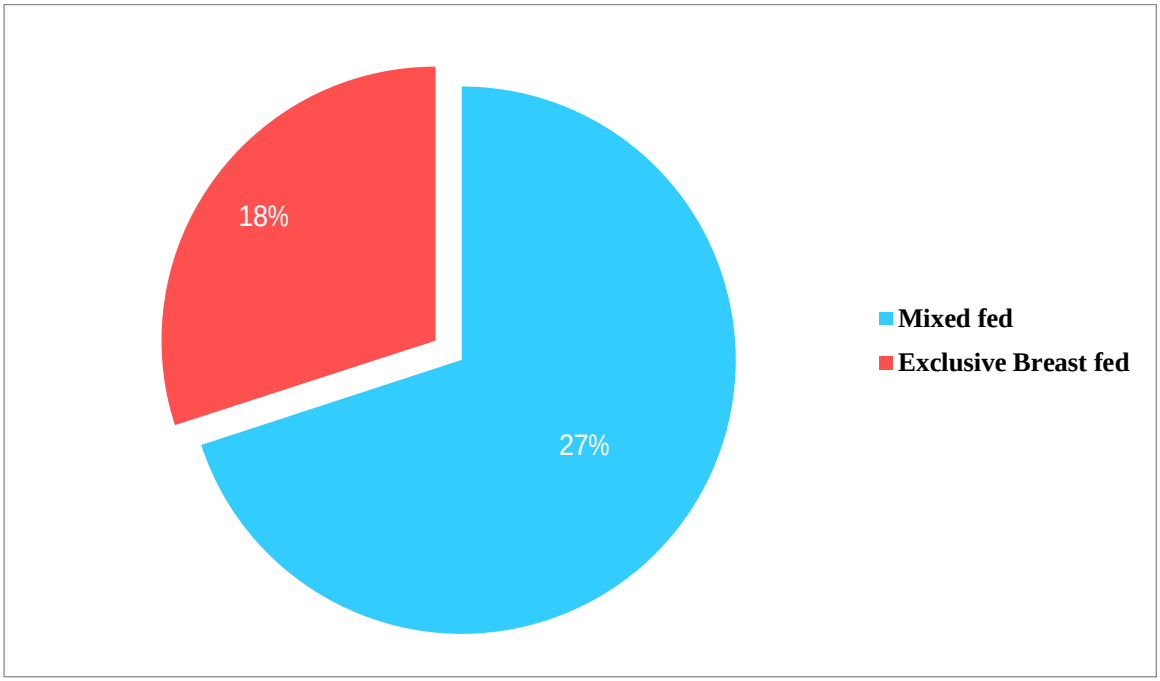


Chart No. 8 Comparison of ELISA and ICT in Detection of Rotavirus Antigen.

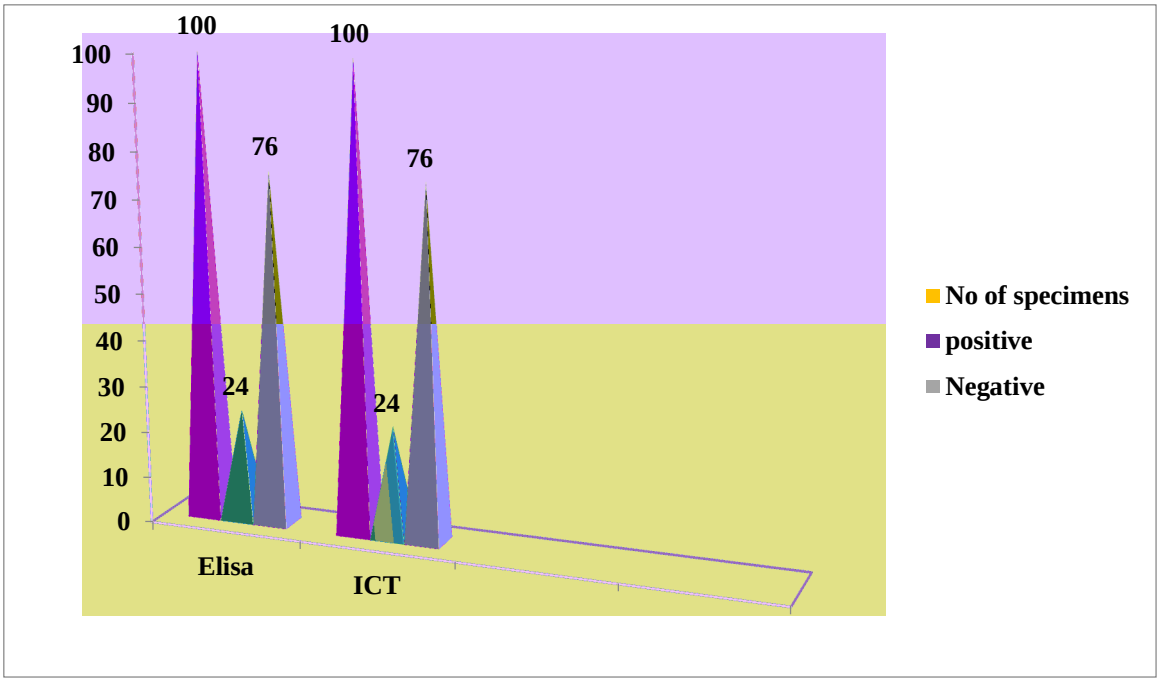
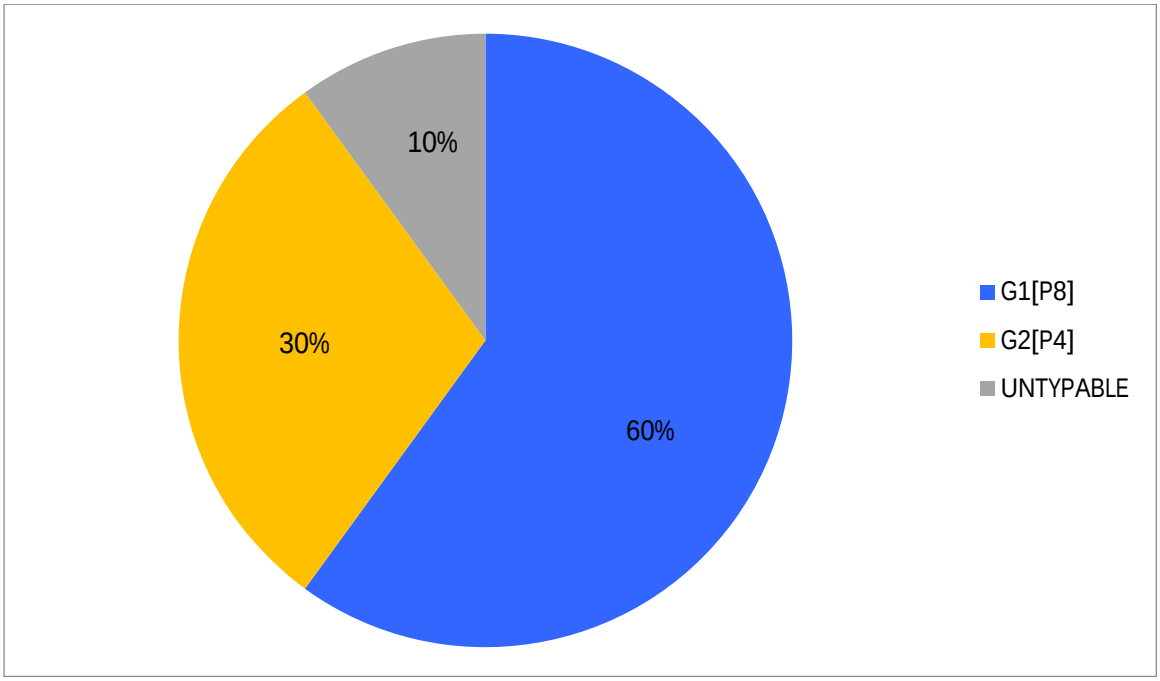


Chart No.9 Genotypic distribution of Rotavirus.



DISCUSSION

DISCUSSION

Diarrhoea continuous to be the significant cause of mortality and morbidity in paediatric population worldwide [98,99]; India accounts for one – fourth of all global diarrhoeal deaths among children less than five years [100]. Even though diarrhoeal deaths have declined considerably through improved access to medical facility, the burden of the diarrhoeal disease continues to remain significant. It is estimated that more than 90% of the diarrhoeal cases occur in developing countries. Globally Rotavirus diarrhoea alone accounts for 125 million cases and more than 6,00,000 deaths every year; of which, India contributes around 22% of deaths, 30% of hospitalization and 8.3% of outpatient visits [94] .

Rotavirus diarrhoea also causes significant economic burden to India which was estimated to be around 4.9 Billion and 5.4 Billion Rupees for hospitalization and outpatient visits respectively each year , whereas the estimated cost to fund a Rotavirus immunization program is even less [104]. The average cost of hospitalization for Rotavirus diarrhoea is approximately from Rs.2000-8400 [105,106] which equates to one or two months of income for an average Indian family.

Rotavirus belongs to the Reoviridae family. Since 1973, when it was first demonstrated in children with acute gastroenteritis [29], numerous studies have been conducted throughout the world regarding the virus and its pathogenesis as well as epidemiology.

Prevalence of Rotavirus:

Global prevalence of Rotavirus gastroenteritis in children below 5 years varies from 6% to 56% whereas in India the incidence of Rotavirus diarrhoea is from 5% to 89.9% [92]. The wide difference in the prevalence of Rotavirus gastroenteritis may be due to difference in the age group, different diagnostic methods conducted, time of onset of disease and seasonal variation of Rotavirus and differences in geographical distribution of Rotavirus among various countries [92].

In this study, the prevalence of Rotavirus infection in children less than five years was 24% which is in accordance with the study by Rahoni SM et al., who reported 22.5% positivity for Rotavirus from stool specimen in children from Brazil [66]. Desai et al., from a hospital based study reported 23.5% of prevalence in children less than five years. It also correlates with the study conducted by Bahl et al., from New Delhi (23.5%) and by Saravanan P et al., from Chennai which showed the prevalence rate of 22.5% in children with acute gastroenteritis. Previous studies across the world revealed different prevalence rates of Rotavirus from 6 to 56% [92]. The prevalence rate of the present study (24%) is within the range of Indian studies, which varies from 5% to 89.9%.

In contrast, a low prevalence of Rotavirus has been reported from Bangalore (16.3%), Kolkata (14.6%) and Vellore (17.8%). This may be related with factors such as good literacy level, better socioeconomic status and good awareness of the people regarding hygiene and sanitary measures.

A high prevalence rate of 89.9% was recorded in Manipur. This may be due to poor literacy level, low socioeconomic status, lack of awareness regarding hygienic practices and sanitary measures. It is also found that high rain fall and low humidity as well as low temperature is associated with high incidence of Rotavirus gastroenteritis [93].

Age distribution of Rotavirus:

In this study, the peak incidence of Rotavirus occurred in the age group of **6 to 12 months** (58.3%) which is closely related with the study by Jain V et al., who reported the prevalence of Rotavirus as highest in the age group between 7 to 12 months; It also correlates with a study by Bahl R et al., in which the result showed the peak incidence of Rotavirus in the age group of 9 to 11 months and a decrease considerably after 18 months of age [71].A hospital based study by Banerjee I et al., reported that the median age of Rotavirus infection in children is 10months (IQR 7.5-12.5) [69].

The higher frequency of Rotavirus diarrhoea in this age group of **6 to 12 months** (58.3%) is because of weaning from breast feed and introduction of

artificial feeds. The chance of infection tends to increase when breast feed is withdrawn since the protective antibodies are lost. Furthermore, introduction of artificial feed is linked with increased risk of infection unless proper hygienic measures are strictly followed.

The **second most common** incidence of Rotavirus was observed in children in the age group of **13 to 24 months** age (25%) as in a study conducted in New Delhi which reported that 98% of hospitalized Rotavirus gastroenteritis cases were below 2 years. The peak incidence of Rotavirus infection was found in children aged 6 to 23 months as reported by Kang G et al., [72]. The higher frequency of Rotavirus infection in this age group is due to various factors like playing with elder siblings or neighbours who may have subclinical infection. Transmission of virus could be possible through fomites such as toys and objects since Rotavirus is stable in room temperature and highly contagious. Even a low **viral load of <100 is enough to cause infection** in susceptible children. Malnutrition is another predisposing factor for these children to develop various infections especially gastroenteritis.

Lesser frequency of Rotavirus incidence was observed in the babies of **0 to 6** months (8.3%) of age which may be due to protection offered by maternal antibodies against Rotavirus infection. The **IgA anti Rotavirus antibody** from mother passes through the breast milk and offers protection to the babies against Rotavirus infection; it does not protect reinfection but gives protection against

severe infection; baby may get subclinical infection without showing any clinical features. Asymptomatic infections in newborn nurseries also occur round the year.

The occurrence of Rotavirus **declined sharply after 2 years** of age (8.3%). This is because of subsequent infections that usually occur less severely due to the antibodies formed against previous exposure to Rotavirus. More than 90% of children aged below 3 years had antibodies against Rotavirus. Every child might have at least one episode of Rotavirus diarrhoea before reaching 5 years [29].

Sex distribution of Rotavirus:

In this study out of 100 children, 24 were positive for Rotavirus antigen of which male children showed 58% (14/24) and female 42% (10/24) positivity. The present study showed that male children were more susceptible for Rotavirus infection than female children (M: F- 1.4:1) which correlates with the study by Banerjee I et al., [69]. Another study by Samir et al., from Bahrain reported a ratio of 1.5:1 in male and female children. A study by Ghazi et al., showed 63% of males and 33% of females were affected by Rotavirus infection [67].

In contrast, no relationship was observed between Rotavirus infection and gender preponderance in a study by Saravanan et al., [65]. It is found that male children excreted a higher rate of Rotavirus in their stool than female children which could be the reason for higher susceptibility of male children. This was also

postulated by Surajudeen A Junaid et al. But the exact reason for susceptibility of male children than female children to Rotavirus infection is obscure and is still under study.

Seasonal trends of Rotavirus:

In this present study, Rotavirus peak occurred during the **cooler months** (September to February) of the year than other months. This study correlated with the study done by Bahl R et al., which also showed, there was a distinct peak of Rotavirus diarrhoea in winter months (November to February) [73]. Another study by Rahoni SM et al., observed a seasonal pattern of Rotavirus diarrhoea in association with cooler months [70]. Shariff M et al., from Eastern Nepal reported that Rotavirus infection occurred round the year with a distinct peak in late winter (January & February) [72].

In contrast, no significant seasonal trends were noted in a study conducted in Vellore by Banerjee I et al., [69]. It is postulated that the reason behind seasonal pattern of Rotavirus infection is because of the possibility of survival of the virus in the environment and its transmission enhanced by low humidity as well as low temperature [29]; higher rainfall also enhances the incidence of Rotavirus infection. It was also noted that, there was no such typical seasonal pattern of Rotavirus in tropical countries due to high temperature and humidity as well as high birth rates [87].

Rotavirus infection in accordance with feeds (Breast feed/Artificial feed)

In this study, lower incidence of gastroenteritis was seen among children **(18%)** who were **exclusively breast fed** (EBF) whereas higher incidence (27%) were found in children who were on mixed feed. This indicates that Rotavirus infection was **less frequent** in breast-fed than babies on mixed feeds because of protection afforded by maternal IgA anti Rotavirus antibodies (80,81,82&83). This is due to the anti-Rotavirus antibody present in the mother because of previous infection; as persistent subclinical Rotavirus infection is common throughout the life in adults. In addition, introduction of supplementary feed increases the chance of infection considerably unless proper hygienic measures such as proper sterilization of feeding bottles/bowls and washing hands with soap and water before preparing/ feeding food are strictly followed.

Rotavirus infection in association with socioeconomic status:

Children from **low socioeconomic status** constitute 80% of the study population and the rest 20% were from middle class in this study. This indicates that these children have a higher risk of diarrhoeal diseases than their counterparts [70,74]. Factors such as, poor sanitation and lack of access to safe drinking water plays a major role in diarrhoel diseases among children less than five years.

Rotavirus infection and Educational Status of parents:

Literacy of the parents also reflects on the health of the children as indicated in our study. Children whose parents had only primary and secondary

level education seem to be affected more by Rotavirus infections [76,77]. The incidence was less among children with parents who were graduates. This could be due to lack of knowledge regarding personal and general hygienic measures as well as teaching hygienic practices to their children [76].

Geographical Distribution of Rotavirus

Despite Rotavirus being prevalent in all geographical areas, the positivity rate seems to be **higher in rural** than urban areas. This is due to lack of awareness among the rural population regarding hygiene and improper waste disposal which are considered as the major risk factors for diarrhoel diseases in children below 5 years. Hand washing before preparing food and feeding their children is also lacking among these population. Open air defecation and vectors such as flies are important environmental issues to be addressed.

Nowadays, there seems to be increasing prevalence of Rotavirus is being noted in urban areas as well. This may be due to early weaning from breast feeding and introduction of artificial feeds, increasing day care centers and play schools where subclinical infections of Rotavirus occur throughout the year and transmission through fomites is also a possibility. Another factor could be children engaging in water sports.

Rotavirus Infection and Water supply

There was a higher frequency of Rotavirus infection in those using public water (25.3%) supply than those used bore wells (20.7%) as evidenced in this study. In developing countries, use of an unsafe drinking water is one of the most important and commonest cause of Rotavirus infections [81,84&85]. Group B Rotavirus is especially known to cause outbreaks of water borne acute gastroenteritis in children and adults in China, India, Bangladesh and Myanmar [95].

Clinical Features in Rotavirus Positive Cases:

Diarrhoea, severe dehydration, vomiting and fever are the common symptoms observed in Rotavirus infected children [8, 86]. In our study, **vomiting was noted in 83 %** (20) of Rotavirus positive children whereas it was 54% (13) in Rotavirus negative cases. Dehydration was a consistent finding in Rotavirus positive cases in this study. **Severe dehydration** was found in **79% of Rotavirus positive cases (19)** while 21% (5) of the children had only mild dehydration. In contrast, severe dehydration was found in **25%** (6) and **mild dehydration in 75%** (18) of **non-Rotavirus gastroenteritis cases**. Fever was found more or less equally in both Rotavirus positive and negative cases.

The above findings from this study indicate that watery diarrhoea, dehydration, fever and vomiting, are consistent symptoms related with Rotavirus

gastroenteritis. Diarrhoea is watery in nature and occurs several times a day which quickly results in severe dehydration particularly, when it is associated with vomiting. But the frequency of diarrhoea and the degree of dehydration are much less in Rotavirus negative diarrhoeal cases. Regarding management, Rotavirus positive cases need supportive measures which include prompt correction of fluid and electrolyte imbalance by ORS/IVF. Appropriate **antibiotics** may be needed in case of bacterial gastroenteritis whereas it should be **avoided in Rotavirus diarrhoea**. So, immense important should be given to diagnose Rotavirus diarrhoea which helps to avoid indiscriminate use of antibiotics in infants and young children.

Comparison of ELISA and ICT in Detection of Rotavirus Antigen from Stool Specimen

Out of 100 stool samples tested for Rotavirus antigen, 24 were positive and 76 were negative in both Enzyme linked immunosorbent assay (ELISA) and Immunchromatography (ICG) methods which indicate both are equally sensitive and specific.

Elisa is the method of choice in diagnosing Rotavirus antigen from stool specimens. It is the most commonly employed method especially when sample load is heavy [87]. Immunochromatography is an equally sensitive and rapid method of Rotavirus diagnosis; it is easy to perform; can be used in places where there is lack of resources and also as a rapid screening test. In addition, it is less

time consuming [88] and does not require any technical expertise. Moreover it seems to be marginally cost effective when compared with ELISA.

Rotavirus Genotyping:

Vast diversity in Rotavirus at genotypic level emphasizes the need for surveillance of circulating Rotavirus strains. Furthermore, surveillance of Rotavirus strains in a particular geographical area is essential to assess the epidemiology of the disease and also to identify the changing pattern following the introduction of vaccination in the community. This study is aimed to find out the prevalence of Rotavirus and its molecular characterization to identify the serotypes which are circulating among the population.

The most common G and P types reported from India are G1,G2,P4,P6 and P8. In Indian children,G9 strains as well as mixed Rotavirus infections are quiet common; in some regions of India, bovine origin of P6 strains have been reported[105]. The unusual Rotavirus strains are formed by re-assortment of human and bovine Rotaviruses which are promoted by the age old tradition of calves and humans living in the same household and socio economic conditions in India. Diversity of Rotavirus strains as well as high prevalence of mixed infections is a distinct feature of Rotavirus epidemiology in India [105].

Out of 24 Rotavirus positive samples (by Elisa method), only 12 were chosen randomly for RT-PCR and VP7(G) and VP4(P) genotyping due to resource

limited settings. Each sample was put in a separate 2 ml sterile centrifuge vial along with viral buffer medium and sent to the Helini Biomolecules, Chennai for RT-PCR and genotyping.

In this study, out of 12 samples tested for RT-PCR, 2samples did not amplify; this may be due to degradation of RNA while transportation or low viral load at the time of specimen collection. The remaining 10 samples were amplified and genotyped in accordance with G (G1,G2,G3,G4 & G9) and P (P4,P6&P8) types. The results showed that G1[P8] was the predominant combination (60%) followed by G2[P4] (30%). This coincides with the study conducted by Girish Kumar, C.P., et al., who reported G1[P8] 62.7% [98].A similiar study from Mumbai by Sushmitha A Shetty et al., reported G1[P8]-33.3% and G2[P4] as 26.7% [99]. It also correlates with results from Karnataka by Ranjitha S. Shetty et al., G1[P8] and G2[P4] as the most common genotypes[92]. S.Babji et al., from Nagercoil reported a prevalence of G1[P8]-44.6% and G2[P4]-18.4% genotypes [100]. The G2[P4] is the predominant combination next to G1[P8] [95,96,97].Out of 10 samples tested for strain characterization, one is un-typable which may be due to mixed infections or point mutation at primer binding site [94,100].

In contrast, a rare emerging strain G11P[25] was isolated from Mumbai during a 2005-2009 study by Sushmitha A. Shetty et al.,[99]. From Pune,G9P[8], G12P[6] and G12P[8] were isolated in 2009 as reported from a study by Shoba D et al.,[94]. Another study from Nagercoil by SBabji et al., reported that G2 [P4]

was predominant than G1[P8] in 2007 and G12P[6] as well as G12P[8] emerged only during that period [100].

In Group A rotavirus, G1 is the most common prevalent strain worldwide. Epidemiologically, Children infected with G1 strain were associated with severe dehydration than those infected with other strains [89,96,97]. Despite the Rotavirus incidence being similar in developed as well as developing countries, more mortalities and health care associated expenses occur in the developing countries [90] mainly because of poor access to medical care, timely intervention, predisposing factors like malnutrition and other social as well as environmental factors.

Rotavirus vaccination:

Interventions such as improved hygiene, sanitary measures and drinking water do not **adequately** prevent the Rotavirus diarrhoea. Other than that there is delay in accessing health care facilities thereby affecting timely intervention and prognosis.So vaccination is the best way of protecting children from Rotavirus infection thereby, reducing disease burden and mortality.

The first vaccine for Rotavirus was licensed for use in United States, Finland and Venezuela in 1998 and it was found to be 80-100% effective in preventing severe group A Rotavirus diarrhoea in children [54]. But it was withdrawn in 1999 by the manufacturer due to the issue of suspected

intussusception in vaccinated children (1in12000). In 2006, two new vaccines, Rotarix and Rotateq were introduced for combating group A Rotavirus which were found to be effective, safe and without risk of intussusception as reported by clinical trials [58]. Mexico was the first among all other countries in the world to use Rotavirus vaccine in children (2006) and it was found that diarrhoea associated deaths decreased by more than 65% [57,58]. Subsequently Rotavirus vaccines were implemented in more than 80 countries around the world and there was found to be a decrease in hospitalization due to diarrhoea reported in more than 10 countries of the developing world[11,12,13,14]. Rotavirus vaccine also significantly reduces hospitalization among older children who were not immunized. This phenomenon is called **indirect effect or herd protection**.

The WHO recommended that Rotavirus vaccination can be integrated in the National Immunization Program (2009) to prevent infant mortality and morbidity [57]. At present, two vaccines - Rotarix ^{TM} (GlaxoSmithkline Vaccines) and Rotateq ^{R} (Merk) have shown good safety profile and excellent efficacy against the globally circulating G (G1,G2,G3 & G4) and P types(P[4], P[6], & P[8]) in large global studies [58].

Integration of these vaccines in National immunization programs has been associated with marked reduction in gastroenteritis related mortality and less frequent diarrhoea related hospitalization as well as outpatient visits in different parts of the world [90&91].

Around 90,000 - 1,53,000 children die from Rotavirus infection each year in India; around **4% of overall mortality in children under five years could be saved** in India, if Rotavirus vaccines were integrated with National Immunization Program [Shaun K Morris et al] (Bulletin of the World Health Organization 2012;90:720-727) [59].

India has developed a new vaccine against Rotavirus - **Rotavac** in May 2013. It was developed under the joint collaboration between India and the United states in the area of medical research. Integration of a Rotavirus vaccine in the National Immunization Program would **prevent more than 25,000 deaths, nearly 3,00,000 hospitalization and more than 6,00,000 outpatient visits** each year in India[105]. On phase 3 clinical trials, Rotavac has showed effective efficacy and excellent safety profile and it would be available at Rs.54 per dose [60]. Indian Academy of Pediatrics has recommended inclusion of Rotavirus vaccine in the National Immunization Program [112].

It is found that the Rotavac reduced severe diarrhoea by more than 56% during first year of life with protection continuing into the second year also. Moreover, the vaccine also exhibited great impact against severe diarrohea of any etiology[5].

In India, Himachal Pradesh, Andhra Pradesh, Haryana and Odisha have become the states involved in pilot study to include Rotavirus vaccination in Universal Immunization Program (UIP). The project was launched in Kangra

district (H.P) by the honorable Chief Minister. It is given as Rotavirus drops(1ml,orally,for two doses-Rotarix and 2ml,orally,for three doses-Rotateq) to babies at 6, 10 and 14 weeks after birth. The vaccine is started at 6 weeks of age and should be completed by 24 weeks of age.

SUMMARY

SUMMARY

- **Rotavirus gastroenteritis** is a global health concern which accounts for around **125 million cases of diarrhea and 6,00,000 deaths** in children under five years worldwide every year.
- The present study was aimed to determine the prevalence of Rotavirus and its molecular characterization in children less than five years with acute gastroenteritis in Coimbatore.
- Overall, 100 children were screened for Rotavirus antigen from stool specimen by Enzyme linked Immunosorbent Assay and Immunochromatography.
- Out of 100 samples, **24 were positive for Rotavirus antigen**; of which, 58 were male children while 42 were females.
- From overall 24 positive samples, 12 were tested for RT-PCR. Of these, 10 samples were PCR confirmed, and were analyzed further for strain characterization and showed **G1P[8]** to be the predominant strain **(60%)** followed by **G2P[4] (30%)** while one sample(10%) was un-typable.
- The Rotavirus **peak** occurred in the age group of **6 to 12 months (58.3%)** followed by **13 to 24 months** (**25%).**
- The majority of cases were observed during the cooler months of the year September to February) although cases were recorded throughout the year.

- The risk factors for Rotavirus diarrhea include artificial feeds, low socioeconomic status and literacy level of parents, open air defecation and use of unsafe drinking water whereas breast feed appears to give protection against Rotavirus.
- Correction of electrolyte and acid base imbalance by Oral Rehydration Solution (ORS)/Intravenous Fluid (IVF) is the mainstay in the treatment of Rotavirus gastroenteritis.
- In Rotavirus, vast **diversity in its genotypic level** emphasizes the significance of epidemiological surveillance of circulating strains.
- The immense importance of **molecular characterization of Rotavirus** is to assess the epidemiology and to detect the **diversity of the current circulating strains** to prepare an effective vaccine to deal with various strains.
- **Rotarix (monovalent)** and **Rotateq (multivalent)** are the effective vaccines against Rotavirus, recommended by the **WHO** currently available worldwide**.**
- **Rotavac** is the indigenous vaccine of **India** as recommended by Indian Academy Of Paediatrics (**IAP**) which shows good safety and excellent efficacy in lowering the disease burden and mortality associated with diarrhea in children less than five years.

CONCLUSION

CONCLUSION

- **Diarrhea** continues to be the **third most common cause of childhood deaths** worldwide; **Rotavirus** remains the leading cause of diarrhea in children less than five years accounting for **6,00,000 deaths** every year
- Out of 100 children tested for Rotavirus antigen from their stool specimens for a period of one year, **24 were positive** which includes 14 male and 10 female children indicating more susceptibility among male children.
- The **maximum Rotavirus positivity** occurred in the age group of **6 to 12 months**.
- The peak incidence was noted during the **cooler months** of the year.
- On screening of Rotavirus antigen from stool specimen, both **ELISA and ICG** showed equal sensitivity and specificity (24 positive and 76 negative).
- ELISA is commonly employed especially when sample load is high while ICG is particularly useful for rapid screening test especially in resource limited settings
- Oral rehydration therapy (low osmolarity ORS) is the mainstay of treatment in Rotavirus Gastroenteritis.
- On molecular characterization of Rotavirus positive samples, **G1 P[8] (60%) and G2 P[4] (30%)** were the most common serotypes isolated in the study.

- **Rotarix (monovalent) and Rotateq (multivalent)** are the universally accepted vaccines against Rotavirus and also recommended by the WHO.
- **Rotavac** is the indigenous vaccine of India which showed excellent efficacy and good safety profile in lowering the illness and death linked with childhood diarrhoea.
- Inclusion of Rotavirus vaccines in the National Immunization Program(NIP) schedule has in Himachal Pradesh , Andhra Pradesh , Haryana and Odisha will go a long way in preventing Rotavirus gastroenteritis and its associated mortality and morbidity in paediatric population.

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ANNEXURES

STATEMENT OF CONSENT

I, Father/Mother/Guardian of _____, do hereby volunteer and consent to participate my ward in this study being conducted by Dr.R. Senthilkumar. I have read and understood the consent form (or) it has been read and explained to me thoroughly. I am fully aware of the study details as well as aware that I may ask questions to him at any time regarding this research on my ward.

Signature / Left Thumb Impression of the Father / Mother / Guardian

Station: Coimbatore

Date:

Signature / Left Thumb Impression and Name of the witness

Station: Coimbatore

Date:

CONSENT FORM

You, _____, aged ____ years, S/o / D/o / _____, residing at _____ _____ are requested to be a participant in the research study titled "**A Study on Rotavirus Gastroenteritis in Children Under Five Years in Coimbatore.**" conducted by Dr.R. Senthilkumar, one of the post graduate trainees in the Dept. of Microbiology, Govt. Coimbatore Medical College and Hospital, Coimbatore. You are eligible for the study as per the inclusion criteria. You can ask him any question or seek from him any clarifications about the study which you may have before agreeing to participate in the study.

ஒப்புதல் படிவம்

நோயாளியின் பெயர் :

வயது :

பாலினம் :

முகவரி :

கோவை அரசு மருத்துவக் கல்லூரியில் நுண்ணுயிரியல் துறையில் பட்ட மேற்படிப்பு பயிலும் மாணவன் மரு. இரா. செந்தில்குமார் அவர்கள் மேற்கொள்ளும் ரோட்டாவைரஸ் கிருமியினால் வரும் வயிற்றுப் போக்கு பற்றிய பல்நோக்கு ஆய்வின் செய்முறை மற்றும் அனைத்து விளக்கங்களையும் கேட்டுக் கொண்டு எனது சந்தேகங்களையும் தெளிவுபடுத்திக் கொண்டேன் என்பதை தெரிவித்துக் கொள்கிறேன்.

நான் இந்த ஆய்வில் முழு சம்மதத்துடனும், சுய சிந்தனையுடனும் கலந்து கொள்ள சம்மதிக்கிறேன்.

இந்த ஆய்வில் என்னை பற்றிய அனைத்து விவரங்கள் பாதுகாக்கப்படுவதுடன் இதன் முடிவு அய்விதழில் வெளியிடப்படுவதில் எனக்கு எந்த ஆட்சேபனையும் இல்லை என்பதை தெரிவித்துக் கொள்கிறேன். எந்த நேரத்திலும் இந்த ஆய்விலிருந்து என்னை விலகிக் கொள்ள எனக்கு முழு உரிமை உண்டு என்பதை அறிவேன்.

இடம் :

தேதி :

கையொப்பம் வயது / கைரேகை

ANNEXURE -I

PROF ORMA

A STUDY ON ROTAVIRUS GASTROENTERITIS IN CHILDREN UNDER FIVE YEARS IN COIMBATORE.

NAME : SAMPLE NO & DATE:

AGE: SEX: M / F IP/OP NO: WEIGHT: Kg

ADDRESS:

SOCIO ECONOMIC STATUS :

WATER SUPPLY :

LITERACY :

TYPE OF FEEDING: BREAST / FORMULA FEEDS / OTHERS

GASTROENTERITIS DATA :

1. VOMITING : YES/NO

2. DIARRHEA : YES/NO IF YES, DURATION –
FREQUENCY

3. DEHYDRATION : YES/NO

4. TEMPERATURE :

5. HOSPITALISED : YES/NO

6. COMPLICATION (If any):

FOR LABORATORY USE:

GROSS EXAMINATION : COLOR - CONSISTENCY

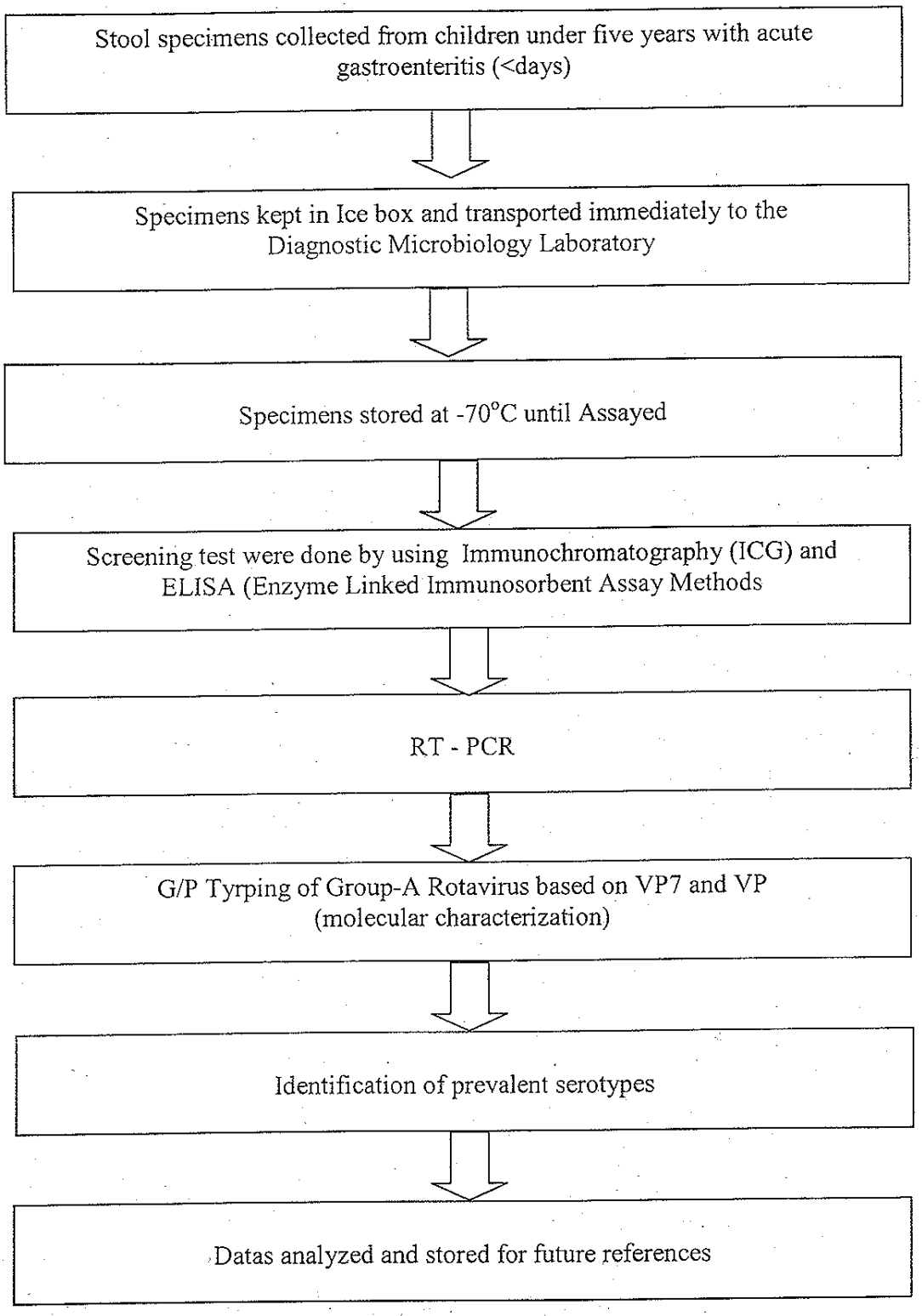
BLOOD : YES/ NO MUCUS: YES/NO

INVESTIGATIONS DONE FOR DETECTION OF ROTAVIRUS ANTIGEN

IMMUNOCHROMATOGRAPHY TEST : POSITIVE/ NEGATIVE

ELISA: POSITIVE/NEGATIVE

FLOW CHART


Stool specimens collected from children under five years with acute gastroenteritis (<days)
Specimens kept in Ice box and transported immediately to the Diagnostic Microbiology Laboratory
Specimens stored at -70°C until Assayed
Screening test were done by using Immunochromatography (ICG) and ELISA (Enzyme Linked Immunosorbent Assay Methods
RT - PCR
G/P Tyrping of Group-A Rotavirus based on VP7 and VP (molecular characterization)
Identification of prevalent serotypes
Datas analyzed and stored for future references

MASTER CHART

MASTER CHART

Sl No.	Name	Age (Months)	Sex	OP/IP No	Month of Illness	ELISA	ICT	Feeds	Geographical area	Socioeconomic Status	Literacy level	Water supply
1	`Varshan	21	M	15713	Jul -15	N	N	MF	U	LS	PE	PW
2	Hariharan	4	M	15718	Jul -15	P	P	BF	R	LS	SE	BW
3	Harshini	16	F	15728	Jul -15	N	N	MF	U	LS	PE	PW
4	Farzan	17	M	15734	Jul -15	N	N	MF	U	LS	SE	PW
5	Kiruthika	29	F	15767	Aug -15	N	N	MF	R	MC	SE	BW
6	Ajmal	15	M	15783	Aug -15	N	N	MF	R	LS	PE	PW
7	Priyanka	13	F	17790	Aug -15	N	N	MF	U	LS	PE	BW
8	Omar Farooq	7	M	15820	Aug -15	P	P	BF	U	LS	SE	PW
9	Rinzal	18	M	15834	Aug -15	N	N	MF	R	LS	SE	PW
10	B/o Uma	1	F	15842	Sep -15	P	P	BF	U	MC	PE	BW
11	Sathis Kumar	36	M	15851	Sep -15	N	N	MF	R	LS	SE	PW
12	Lakshitha	16	F	15862	Sep -15	N	N	MF	R	LS	PE	PW
13	Ajay	60	M	15874	Sep -15	N	N	MF	U	LS	SE	PW
14	Divya Sree	9	F	15879	Sep -15	P	P	MF	R	MC	PE	BW

15	Poornima	35	F	15895	Sep -15	N	N	MF	U	LS	SE	PW
16	Aarthi	14	F	15909	Sep -15	N	N	MF	U	MC	PE	BW
17	Naveen Kumar	16	M	15917	Oct -15	N	N	MF	U	LS	SE	PW
18	Anu Shri	48	F	15924	Oct -15	N	N	MF	R	LS	PE	PW
19	Raghav	7	M	15933	Oct -15	P	P	BF	U	LS	SE	BW
20	Kavin	5	M	15946	Oct -15	N	N	BF	R	LS	PE	PW
21	Kavya Sree	8	F	15957	Oct -15	N	N	BF	U	MC	PE	PW
22	Sri Ram	10	M	15966	Oct -15	P	P	BF	U	LS	SE	PW
23	Thanga Pandi	23	M	15974	Oct -15	N	N	MF	U	MC	PE	PW
24	Anushya	8	F	15983	Oct -15	P	P	BF	R	LS	SE	BW
25	Vishnu	43	M	15987	Oct -15	N	N	MF	U	LS	PE	BW
26	Janani	12	F	16011	Nov -15	P	P	MF	U	LS	SE	PW
27	Manoj	8	M	16017	Nov -15	P	P	BF	R	LS	SE	BW
28	Rekha	44	F	16022	Nov -15	N	N	MF	U	MC	PE	PW
29	Kamna	18	F	16036	Nov -15	N	N	MF	U	LS	G	BW
30	Kamalesh	5	M	16039	Nov -15	N	N	BF	U	LS	PE	PW
31	Kanika	11	F	16044	Nov -15	P	P	MF	R	LS	SE	BW
32	Madhumitha	2	F	16056	Nov -15	N	N	BF	U	MC	SE	PW

33	Mohammed Khan	56	M	16063	Nov -15	N	N	MF	U	LS	PE	BW
34	Lakshman	12	M	16069	Nov -15	N	N	MF	R	LS	SE	PW
35	Mukesh Kumar	26	M	16074	Nov -15	N	N	MF	U	LS	PE	BW
36	Suganya	12	F	16080	Nov -15	N	N	MF	U	MC	PE	PW
37	Swetha	19	F	16097	Nov -15	N	N	MF	R	LS	SE	PW
38	Naveen	7	M	16122	Dec -15	P	P	BF	U	LS	SE	BW
39	Meena	5	F	16119	Dec -15	N	N	BF	U	LS	G	BW
40	Kiran	32	M	16131	Dec -15	N	N	MF	U	LS	PE	PW
41	Guru	15	M	16145	Dec -15	N	N	MF	R	LS	SE	BW
42	Anwar	19	M	16167	Dec -15	N	N	MF	U	MC	SE	
43	Saranya	8	F	16178	Dec -15	P	P	BF	R	LS	SE	PW
44	Prasath	11	M	16189	Dec -15	N	N	MF	U	LS	PE	PW
45	Devaraj	10	M	16191	Dec -15	N	N	MF	U	MC	SE	BW
46	Shruthi	17	F	16213	Dec -15	N	N	MF	R	LS	SE	PW
47	Kamala Kannan	6	M	16216	Dec -15	N	N	BF	U	LS	PE	PW
48	Raveena	13	F	16225	Dec -15	P	P	MF	U	LS	SE	PW
49	Lakshmi	10	F	16237	Dec -15	N	N	MF	U	LS	PE	BW

50	Joel Sharon	9	M	16244	Dec -15	N	N	BF	R	LS	SE	PW
51	Sri Nithi	7	F	16258	Dec -15	N	N	MF	U	MC	SE	PW
52	Raghu	18	M	16267	Dec -15	P	P	MF	R	LS	PE	PW
53	Ramraj	2	M	16273	Jan -16	N	N	BV	U	LS	SE	BW
54	Kalaiselvi	9	F	16289	Jan -16	N	N	BF	R	LS	SE	PW
55	Krishnan	8	M	16299	Jan -16	N	N	BF	U	MC	PE	BW
56	Nisha	16	F	16313	Jan -16	P	P	MF	U	LS	SE	PW
57	Ramar	14	M	16325	Jan -16	N	N	MF	R	LS	PE	BW
58	Raja	3	M	16332	Jan -16	N	N	BF	U	LS	G	PW
59	Alageshwari	60	F	16347	Jan -16	P	P	MF	R	MC	SE	PW
60	Ikbal	11	M	16356	Jan -16	N	N	MF	U	LS	PE	BW
61	Prema	6	F	16364	Jan -16	N	N	BF	U	LS	SE	PW
62	Rupa	48	F	16377	Jan -16	N	N	MF	R	LS	SE	PW
63	Ferros Kapoor	20	M	16380	Jan -16	N	N	MF	R	MC	PE	PW
64	Abishek	10	M	16389	Jan -16	P	P	MF	U	LS	SE	BW
65	Rohith	19	M	164o1	Jan -16	P	P	MF	U	LS	PE	PW
66	Aishwarya	9	F	16414	Jan -16	N	N	BF	R	MC	SE	PW
67	Mohammed Rafiq	42	M	16425	Jan -16	N	N	MF	R	LS	SE	BW

68	Dinesh	9	M	16436	Jan -16	N	N	BF	U	LS	PE	PW
69	Ananya	4	F	16444	Jan -16	N	N	BF	R	LS	SE	PW
70	Gokul	48	M	16458	Feb -16	P	P	MF	U	LS	PE	PW
71	Sujitha	21	F	16469	Feb -16	N	N	MF	U	LS	SE	PW
72	Gowtham	17	M	16483	Feb -16	P	P	MF	R	LS	SE	PW
73	Azar	4	M	16496	Feb -16	N	N	BF	U	LS	PE	PW
74	Vishal	8	M	16512	Feb -16	N	N	MF	U	MC	SE	BW
75	Deeptha	8	F	16526	Feb -16	N	N	MF	R	LS	SE	PW
76	Deepan	11	M	16534	Feb -16	N	N	MF	U	LS	PE	BW
77	Mukil	9	M	16544	Feb -16	N	N	BF	U	LS	SE	PW
78	Manoj	14	M	16559	Feb -16	N	N	MF	R	LS	G	PW
79	Jeevitha	11	F	16567	Mar -16	N	N	MF	U	MC	PE	PW
80	Aadhav	5	M	16573	Mar -16	N	N	BF	U	LS	SE	BW
81	Vinothini	10	F	16582	Mar -16	N	N	MF	R	LS	SE	PW
82	Pradeepa	36	F	16590	Mar -16	N	N	MF	R	LS	SE	PW
83	Kishore	12	M	16606	Mar -16	P	P	MF	U	LS	PE	PW
84	Ashwin	40	M	16613	Apr -16	N	N	MF	U	LS	PE	BW
85	Priya	18	F	16620	Apr -16	N	N	MF	U	LS	SE	PW

86	Sachin	48	M	16633	Apr -16	N	N	MF	R	LS	SE	BW
87	Varun	8	M	16642	Apr -16	N	N	BF	U	LS	PE	PW
88	Bhoomika	7	F	16656	Apr -16	P	P	BF	U	LS	SE	BW
89	Pranav	36	M	16667	Apr -16	N	N	MF	R	LS	SE	PW
90	Hashini	28	F	16678	May -16	N	N	MF	U	LS	SE	PW
91	Jamuna	10	F	16689	May -16	N	N	BF	R	MC	PE	BW
92	Yuvraj	7	M	16702	May -16	N	N	BF	U	LS	SE	PW
93	Vidhya	30	F	16718	May -16	N	N	MF	U	LS	PE	BW
94	Nikhil	23	M	16721	May -16	P	P	MF	R	LS	SE	PW
95	Bharat	8	M	16734	Jun -16	N	N	BF	R	LS	SE	BW
96	Poorvi	11	F	16746	Jun -16	N	N	MF	R	LS	PE	PW
97	Dhivakar	30	M	16759	Jun -16	N	N	MF	U	LS	SE	PW
98	Lavanya	8	F	16763	Jun -16	P	P	BF	R	MC	G	BW
99	Harshitha	15	F	16774	Jun -16	N	N	MF	U	LS	SE	PW
100	Ishanth	10	M	16783	Jun -16	N	N	BF	R	LS	PE	PW

KEY TO MASTER CHART

ELISA	Enzyme Linked Immunosorbent Assay
ICG	Immunochromatography
N	Negative
P	Positive
MF	Mixed Feed
BF	Breast Feed
U	Urban
R	Rural
LS	Low Socio Economic Status
MC	Middle Class
PE	Primary Education
SE	Secondary Education
G	Graduates
PW	Public Water
BW	Bore wells